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ABSTRACT BOOK

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A001

APTAMER-BASED THERAPEUTICS TARGETING AXL AND CAFS TO OVERCOME CHEMORESISTANCE IN LUNG CANCER

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INTRODUZIONE

Chemoresistance remains a major obstacle in the treatment of non-small cell lung cancer (NSCLC), critically limiting therapeutic efficacy. AXL receptor overexpression is strongly implicated in epithelial–mesenchymal transition (EMT), pro-survival signaling and resistance phenotypes. In parallel, the tumor-suppressive miRNA let-7a-5p is frequently downregulated in NSCLC and orchestrates key oncogenic networks. Here, we exploited a rational nucleic acid based strategy to overcome resistance by targeting AXL with the GL21 aptamer alone or in combination with let-7a-5p in a chimeric construct.

METODI

Resistant NSCLC models were established by IC_{50} validation. AXL expression was confirmed prior to intervention. Cells were treated with optimized doses of GL21, GL21–let-7a-5p chimera and cisplatin alone or in combination. Cell viability was quantified and apoptotic signaling was assessed via Bax/Bcl-2 ratio. DNA damage induction was evaluated by γ-H2AX expression and confirmed by PI fluorescence microscopy. Docking assessed GL21 binding to AXL in both unconjugated and miRNA-conjugated forms. Let-7a-5p target genes were identified through validated databases and analyzed for expression and co-expression in NSCLC datasets. Data were validated by colony formation assays, qPCR of target suppression, 3D tumor models and CAF-derived system.

RISULTATI

The GL21–let-7a-5p chimera markedly potentiated cisplatin sensitivity compared to monotherapy. Combination treatment significantly reduced viability increased the Bax/Bcl-2 ratio and enhanced γ-H2AX accumulation, indicating amplified DNA damage and apoptosis. Fluorescence imaging confirmed increased membrane permeability consistent with cell death. Bioinformatic analysis revealed elevated expression and coordinated co-expression of let-7a-5p targets in NSCLC, supporting their functional relevance. Docking studies demonstrated stable GL21–AXL interaction, with preserved receptor engagement following miRNA conjugation.

CONCLUSIONI

Simultaneous AXL targeting and let-7a-5p restoration synergistically enhance cisplatin efficacy by reactivating apoptotic pathways and promoting DNA damage in resistant NSCLC. This rationally engineered nucleic acid based combinatorial strategy offers a translationally promising approach to overcome chemoresistance.

A002

CBX3 AS A PROGNOSTIC BIOMARKER IN LUNG CANCER

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INTRODUZIONE

Lung cancer is among the most common and aggressive malignancies worldwide, marked by a poor prognosis and high mortality. Advances in understanding its molecular mechanisms have highlighted the significance of chromatin-modifying proteins, which are key regulators of gene expression and cellular function. Among these, Chromobox protein 3 (CBX3) has emerged as a potential epigenetic biomarker in lung cancer.

METODI

A549 and NCI-H1975 cells were cultured in standard conditions. We used molecular and cellular assays to test CBX3 knockdown and overexpression effects on proliferation and survival. Gene expression and epigenetic profiling identified CBX3-linked regulatory pathways. High-throughput screening found compounds that modulate CBX3 activity and block its tumor-promoting effects. Cell viability assay was performed with CCK-8. Basal expression was performed by Western blot and RT-PCR.

RISULTATI

In silico data have indicated high expression of CBX3 in almost all cancer tissues, including lung cancer, and such high expression is associated with a worse prognosis. Notably, RNA sequencing data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression portal (GTEx) show that lung tissues express CBX3 at a significantly higher level than normal lung tissues. Western blot and RT-PCR analyses revealed higher expression of CBX3 in the lung cancer cell lines A549 and NCI-H1975, compared to the normal human lung fibroblast cell line MRC-5. From tissue microarray analysis containing 120 lung tissues samples, CBX3 staining in lung tissues was mainly nuclear. In particular, CBX3 expression is strongly associated with tumor grade, higher histological grade, clinical stage, and tumor size.

CONCLUSIONI

Deciphering the role of CBX3 in lung carcinogenesis could clarify the molecular mechanisms driving tumor aggressiveness. Its identification as a biomarker offers a compelling approach for targeted therapies. Furthermore, the link between CBX3 levels and clinical outcomes underscores its prognostic value, suggesting that inhibiting its function could be a promising treatment plan.

A003

MLH1 PROMOTER METHYLATION, MISMATCH REPAIR, AND INTESTINAL DIFFERENTIATION MARKERS IN PAPILLARY THYROID CARCINOMA

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INTRODUZIONE

Papillary thyroid carcinoma (PTC) is the most common subtype of thyroid malignancy and is generally associated with a favorable prognosis. However, a subset of these tumors exhibits more aggressive behavior, underscoring the need for refined molecular and immunohistochemical characterization to improve diagnostic precision and prognostic stratification. Given the shared embryologic origin of the thyroid gland and gastrointestinal tract from the endoderm, intestinal differentiation markers (CDX2 and SATB2) and alterations in DNA mismatch repair (MMR) pathways, including MLH1 promoter methylation, may represent biologically relevant features in thyroid tumors. Their role in PTC, however, remains unclear.

METODI

This study performed MLH1 promoter methylation analysis by pyrosequencing and protein expression of MLH1, PMS2, MSH2, MSH6, SATB2, and CDX2 by immunohistochemistry in 63 PTCs. Clinicopathological and molecular data were also collected for comparative analyses and correlation with progression-free survival (PFS).

RISULTATI

All PTCs retained expression of MMR proteins (MLH1, PMS2, MSH2, and MSH6), including the three tumors with MLH1 promoter hypermethylation. CDX2 positivity was detected in only two (3.2%) PTCs (classic and follicular subtypes), and SATB2 expression was absent in all cases. No significant association was identified between MLH1 promoter methylation, MMR protein expression, CDX2 or SATB2 expression, and PFS. In contrast, venous invasion was confirmed as a significant predictor of worse PFS (HR = 3.34; 95% CI 1.27–8.76; p = 0.014) in univariate analysis.

CONCLUSIONI

MLH1 promoter methylation, MMR deficiency, and CDX2 are rare events in PTC and do not appear to influence PFS. Although infrequent, CDX2 expression is not exclusive to any PTC subtype.

A004

DISSECTING METTL3 INHIBITION AS A POTENTIAL PLATFORM FOR SYNTHETIC LETHALITY IN NSCLC

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INTRODUZIONE

N6-methyladenosine (m6A) is the most prevalent internal mRNA modification in eukaryotes, dynamically regulated by writers, readers, and erasers. m6A dysregulation promotes oncogenic translation, proliferation, and therapy resistance in lung cancer. NSCLC remains a leading cause of cancer related mortality, with frequent recurrence, metastasis, and therapy resistance contributing to poor patients outcomes. Single agent therapies, including chemotherapeutics, often fail due to DNA repair plasticity. After testing several chemotherapeutic agents, we identified rucaparib as a potential candidate for combination with METTL3 inhibition. This study investigates whether METTL3 inhibition(STC-15) creates synthetic lethality with chemotherapeutics and PARPi across genetically distinct NSCLC models.

METODI

NSCLC lines A549, NCI-H1975, NCI-H1299 and normal BEAS-2B cells were treated with STC-15, cisplatin, doxorubicin, temozolomide (TMZ), paclitaxel, or rucaparib. Viability was measured by CCK-8, colony formation unit (CFU) assay, and proliferation assays, while migration activity was assessed via scratch assay. Bioinformatic analyses using DepMap and GEPIA were conducted to assess METTL3 dependency across NSCLC cell lines and to evaluate correlation between METTL3 and PARP expression in lung cancer.

RISULTATI

STC-15 slightly reduced viability in NSCLC versus normal cells. Among chemotherapies, rucaparib showed reproducible synthetic lethality with STC-15. In both A549 and NCI-H1299 cells, STC 15 combined with rucaparib significantly reduced viability ($p<0.005$, $n=3$), which was further validated by phenotypic assays including scratch wound healing, CFU, and time dependent proliferation assays.

CONCLUSIONI

These findings establish METTL3 inhibition as a synthetic lethality platform in NSCLC. The selective synergy between METTL3i and PARPi across genetically distinct models provides rationale for combining epitranscriptomic targeting with PARP inhibition. This strategy overcomes the limitations of single agent therapies and offers a potential possibility to delay or prevent acquired resistance. Further insights from ongoing RNA-seq and molecular pathways studies will guide clinical translation of this combination for NSCLC patients. Thus, targeting METTL3 together with PARP provides a clinically translatable approach to combat NSCLC therapy resistance.

A005

KIRCONG CHIM PEG ENHANCES VORINOSTAT EFFICACY IN TNBC BY TARGETING STAT3 SIGNALLING

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INTRODUZIONE

Triple negative breast cancer (TNBC) is a highly aggressive type of cancer with poor clinical outcome. Evidence suggests that the inflammatory signaling pathway IL-6/JAK/STAT3 may attenuate the anti-cancer activity of histone deacetylase inhibitors (HDACi) by promoting survival in TNBC. We tested the HDACi suberoylanilide hydroxamic acid (SAHA, Vorinostat) for its anti-cancer effects in combination with a SOCS3 peptidomimetic, KIRCONG chim PEG designed to inhibit STAT3 phosphorylation (pSTAT3) in TNBC cells.

METODI

SOCS3 expression was evaluated in MDA-MB-231, and MDA-MB-468 cells by western blot. Cells were treated with vorinostat for 72h to evaluate survival by MTT and trypan blue exclusion assays and immunogenic cell death (ICD) hallmarks including ATP release measured by luminescence-based assay; calreticulin surface exposure, and HMGB1 levels analyzed by flow cytometry. IL-6 levels were assessed by ELISA. In cells co-treated with vorinostat and KIRCONG chim PEG, p-STAT3, BAK, cleaved caspase-3 and COX-2 expression were evaluated by western blot. Immune cell activation was evaluated by 48h incubation of freshly isolated PBMCs with the conditioned media (CM) collected from co-treated MDA-MB-231 cells. HLA-DR and CD69 were evaluated within CD4+ and CD8+ T cell subsets by flow cytometry.

RISULTATI

Low basal levels of SOCS3 in MDA-MB-231 compared to MDA-MB-468 cells were associated with the higher IL-6 secretion observed in MDA-MB-231 cells. Although vorinostat reduced cell survival and induced ICD markers (ATP release, calreticulin surface exposure, and increased HMGB1 levels) in MDA-MB-231 cells, it up-regulated IL-6 favoring a persistent STAT3 phosphorylation. In combination with KIRCONG chim#PEG, vorinostat reduced pSTAT3 and increased BAK, and cleaved caspase3 expression. Vorinostat increased COX-2 expression, while a trend toward reduction was observed upon combinatorial treatment, suggesting a potential modulation of inflammatory signaling. CM from co-treated MDA-MB-231 cells promoted CD4+T cell activation increasing HLA-DR and CD69 expression.

CONCLUSIONI

Functional replacement of SOCS3 represents a promising therapeutic strategy to limit IL-6/JAK/STAT3 signaling and potentiate the anti-cancer and immunogenic effects of HDACi in IL-6 high TNBC cells.

A006

PREP1 DEFICIENCY PROTECTS FROM HYPERGLYCEMIA-INDUCED RETINAL NEURODEGENERATION

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INTRODUZIONE

Hyperglycemia is one of the main risk factors contributing to the onset of diabetic retinopathy (DR) and one of the most serious diabetic complications, capable of partially or completely compromising vision. In this context, neuroinflammation is considered a key mechanism in the pathogenesis of early DR, although the molecular factors regulating these processes are not yet fully defined. For this reason, it is of particular interest to identify the possible molecular mediators that regulate these processes.

Prep1 is a ubiquitously expressed homeodomain transcription factor that impairs glucose and lipid homeostasis in insulin-target tissues, and Prep1 hypomorphic heterozygous mice ($Prep1^{i/+}$) expressing low levels of protein are protected from streptozotocin (STZ)-induced diabetes. In this study, we have evaluated the role of Prep1 in the adult mouse retina.

METODI

Hyperglycemia was induced by intraperitoneally injecting low doses of STZ in WT and in $Prep1^{i/+}$ for 5 consecutive days. The effects of hyperglycemia on retinal morphology were analyzed by hematoxylin and eosin staining. Additionally, a multiplex cytokine analysis was performed on serum samples from STZ-treated WT and $Prep1^{i/+}$ mice using a multiplex assay

RISULTATI

$Prep1^{i/+}$ mice showed a rapid reduction in blood glucose, maintained up to 12 weeks after STZ treatment, compared to WT mice. At week 12, $Prep1^{i/+}$ mice showed reduced retinal damage, including less ganglion cell layer disorganization and less vacuolar ganglion cell degeneration compared to WT mice. Furthermore, less disruption of the inner and outer nuclear layers was observed in $Prep1^{i/+}$ mice, accompanied by less pronounced edema and remodeling.

In parallel, a cluster of proinflammatory cytokines appeared globally reduced in $Prep1^{i/+}$ mice compared with WT littermates following STZ administration. Among the analysed mediators, $IFN\gamma$ and $TNF\alpha$ levels were significantly lower in $Prep1^{i/+}$ mice than in WT mice.

CONCLUSIONI

These results indicate that Prep1 silencing prevents hyperglycemia-induced retinal neurodegeneration in adult mice by reducing the inflammatory response.

A007

CHALLENGES POSED BY EU EVDR REGULATIONS FOR PATHOLOGY LABORATORIES AND COMPLIANCE SOLUTIONS

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INTRODUZIONE

The EU IVDR regulation are mainly directed towards manufacturers of IVD systems. Many pathological laboratories in Europe are now considered manufacturers because they develop in-house tests. This is placing a great strain on lab personnel and quality managers. The College of American Pathologists (CAP) as the world's largest organization devoted to quality management in the medical laboratory has been tasked by the FDA to develop meaningful and enforceable directives to maintain quality for in-house tests. These solutions for compliance should be able to satisfy the needs of IVDR compliance.

METODI

We have analyzed the requirements in IVDR and compared to the CAP's requirements for accredited labs as laid down in the proprietary checklist documents. The CAP's checklists are used to assess a lab striving for CAP accreditation. For the purpose of this study, we have concentrated on the three common and two discipline-specific checklists. Each checklist contains a collection of risk scenarios that could impact the quality of the laboratory's end result and suggests possible compliance solutions.

RISULTATI

The IVDR regulations state that a test is lab-derived if:
- The commercially individually available components are used on a protocol developed by the lab or
- A commercially available assay system is modified beyond the manufacturer's recommendations in its instructions for use

We have concentrated on comparing the CAP's requirements for accredited labs in the areas of
- Method development and validation: The CAP's checklist items Validation Study Approval, Validation Studies-Specimen Types, Manufacturer's Instructions, Verification of Test Performance Specifications, Validation of Test Performance Specifications, Clinical Claims Validation, Test List, Calibration and Quality Control Procedures,
- Creation of a quality management system: checklist points QM Program, Risk Assessment, Ongoing Quality Assessment Monitoring, Procedure Manual,
- Document management and retention: checklist points Document Control, Record/Specimen Retention,
- Post-market surveillance/Reporting: checklist points Corrective and Preventive Action, Adverse Patient Event Reporting

CONCLUSIONI

The CAP's accreditation checklists provide a detailed blueprint for a high-quality laboratory and cover the IVDR requirements for in-house tests.

A008

EVALUATION OF NOVEL COMPOUNDS FOR REDUCTION OF SERUM BILIRUBIN.

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INTRODUZIONE

Hyperbilirubinemia is a clinically relevant condition observed in neonatal hypoxia, cirrhosis, hepatitis, liver tumors, hemolytic disorders, and drug-induced hemolysis. Unconjugated bilirubin is poorly water-soluble, tightly bound to albumin, and cannot be directly excreted by the kidneys, Bilirubin accumulation, when production exceeds hepatic conjugation capacity, is associated with significant adverse effects, particularly neurotoxicity in neonates and potential multi-organ dysfunction including kidney. Despite its clinical relevance, there is currently no specific pharmacological approach that directly reduces circulating bilirubin levels.

METODI

We analyzed serum samples from 15 patients with hyperbilirubinemia (1.30–6.10 mg/dL), incubated for 24 hours with cyclodextrin-based compounds (Ciclodestrin control, SG-1087, SG-1099) at different concentrations. Bilirubin levels were quantified using a Bilirubin Assay Kit (Sigma-Aldrich). In vivo, six mice bearing gastric carcinoma xenograft models were treated intraperitoneally with the same compounds, using doses selected based on IC50 values. After 24 hours, serum and urine were collected and analyzed for bilirubin content.

RISULTATI

For in vivo evaluation, compounds were tested in a gastric carcinoma mouse model using doses selected based on in vitro IC50 values. Compared with untreated controls, treated mice showed a marked reduction in circulating bilirubin, with an approximately 10-fold decrease in serum levels, accompanied by a concomitant increase in urinary bilirubin excretion, indicating enhanced systemic clearance of unconjugated bilirubin. Among the tested compounds, SG-1099, conjugated with mitochondrial carbonic anhydrase XII (CA XII), showed the highest efficacy both in vitro and in vivo. However, its pharmacokinetic properties, tissue distribution, and bioavailability have not yet been characterized, and optimal routes of administration remain to be determined.

CONCLUSIONI

Despite these limitations, the present findings support SG-1099 as a promising candidate for further development.

A009

THE AKAP1 RIBONUCLEOPROTEIN NETWORK INTEGRATES MITOCHONDRIAL HOMEOSTASIS, P-BODY DYNAMICS AND PROTEIN TRANSLATION IN CYCLING CELLS.

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INTRODUZIONE

Integration of protein translation and mitochondrial activities represents a mechanism to respond to specific cellular needs. During cell division, mitochondrial dynamics, oxidative phosphorylation and protein synthesis are coordinated to allow the progression of cell cycle. AKAP1, is a mitochondrial AKAP that targets PKA to the outer mitochondrial membrane (OMM). AKAP1 contains at its C-terminal core a K-Homology domain (KH) that binds nuclear-transcribed mRNAs encoding for mitochondrial proteins, facilitating their translation and import into the organelle.
Physical and functional communications between mitochondria and other cellular compartments contribute to regulate the protein translation. The temporary storage of mature mRNAs within non-membranous ribonucleoprotein (RNP) cytoplasmic condensates, namely P-bodies, represents a conserved mechanism that finely controls the translation repression of selected classed of mRNAs, adapting the cellular proteome to stress conditions or specific metabolic needs.
P-bodies RNA composition and dynamics differentially change during cell cycle, indicating the existence of a mechanism that controls cell cycle-dependent condensation/fate of selected classes of mRNAs.
The aim of the study is to show how during the different phases of cell cycle, AKAP1 functions as a metabolic sensor that efficiently links oxidative metabolism to P-body dynamics and protein translation, ensuring the cell cycle progression.

METODI

Integrating transcriptomic analysis, proximity labelling assay, metabolic analysis and confocal microscopy, we investigated the role of AKAP1 in the regulation of protein translation in course of cell cycle progression of tumoral cells.

RISULTATI

Here, we report the identification of a molecular network assembled by AKAP1 at mitochondria that includes mRNAs, components of the P-bodies (DDX6 and EDC3) and mitotic kinases (CDK1/2). During the interphase, AKAP1 dynamically coordinates cAMP signaling, mitochondrial metabolism, P-body dynamics and protein translation to ensure the proper cell cycle progression. At mitosis, CDK-induced proteolysis of AKAP1 ,via a cullin-mediated ubiquitin pathway, reduces oxidative metabolism, promotes mitochondrial fission and reshapes mRNA translation, finalizing the cell cycle. Disruption of this network impairs cell cycle progression.

CONCLUSIONI

These findings unveil a regulatory mechanism controlled by AKAP1 that functionally and dynamically links oxidative metabolism to protein synthesis in cycling cells.

A010

FUNCTIONAL CHARACTERIZATION OF CBX2 INHIBITION IN GBM

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INTRODUZIONE

Polycomb group (PcG) proteins regulate epigenetic silencing and play key roles in development and lineage specification. Dysregulation of PcG components, including members of the Chromobox (CBX) family, is increasingly linked to oncogenesis. CBX2, the canonical chromatin reader of PRC1, has recently emerged as a potential oncogenic driver. Preliminary silico analyses indicate that CBX2 is markedly upregulated in glioblastoma (GBM), the most aggressive primary brain tumor in adults.
This study aims to functionally characterize CBX2 in GBM by integrating reverse-genetic perturbations, phenotypic assays, and multi-omic profiling to elucidate its biological role and assess its potential as a therapeutic target.

METODI

Differential expression analyses using TCGA and CGGA datasets revealed consistent upregulation of CBX2 in GBM relative to non-tumor controls. DepMap expression profiling was subsequently used to identify the most appropriate cellular models for functional studies. CBX2 expression levels were validated by qPCR and Western blot in SVGp12, U87, U138, and T98G cells, confirming low expression in U87, intermediate expression in U138, and high expression in T98G. Based on these profiles, U87 cells were selected for CBX2 overexpression using a GFP-tagged recombinant construct, whereas T98G cells were chosen for CBX2 knockdown via lentiviral shRNA vectors. Perturbation efficiencies were verified by qPCR and Western blot. The engineered cell lines were subsequently characterized through morphological assessment, viability, proliferation assays and colony formation assays.

RISULTATI

CBX2 knockdown in T98G cells induced marked morphological stress, followed by a rapid decline in viability and proliferative capacity. CCK-8 assays showed a >50% reduction in proliferation at all measured time points, accompanied by a loss of clonogenic potential. In contrast, CBX2 overexpression in U87 cells impaired both proliferation and colony formation, and unexpectedly also increased endogenous CBX2 expression, suggesting the presence of a potential positive autoregulatory feedback loop.

CONCLUSIONI

Collectively, these findings support a pro-oncogenic role for CBX2 in GBM, with its modulation exerting robust and directionally consistent effects on tumor cell growth. Additional molecular and multi-omic analyses are underway to delineate the pathways governed by CBX2 activity.

A011

INTEGRATED TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REMODELING OF ADIPOSE TISSUE BY THE DPP-4 INHIBITOR LINAGLIPTIN IN EXPERIMENTAL OBESITY

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INTRODUZIONE

Dipeptidyl peptidase-4 inhibitors (DPP-4i) are widely used for the treatment of type 2 diabetes mellitus and are generally considered weight-neutral agents, as they do not induce clinically significant reductions in body weight or fat mass. Nevertheless, DPP-4 inhibition improves adipose tissue function and reduces obesity-associated inflammation. The molecular mechanisms underlying this dissociation between metabolic improvement and lack of weight loss remain poorly understood. We investigated whether the DPP-4 inhibitor linagliptin modulates adipogenic and metabolic pathways in visceral adipose tissue (VAT) of high-fat diet–fed mice through coordinated mRNA and microRNA regulation.

METODI

Male C57BL/6N mice were fed for 15 weeks with normal chow diet, high-fat diet (HF), or HF supplemented with linagliptin (120 µg/day). VAT was analyzed using targeted qPCR arrays for adipogenesis-related transcripts and diabetes-associated microRNAs. Bioinformatic analyses included protein–protein interaction networks, Gene Ontology enrichment, and in silico miRNA target prediction.

RISULTATI

HF induced transcriptional and miRNA signatures consistent with impaired adipogenesis, adipocyte hypertrophy, inflammation, and metabolic dysfunction. Linagliptin partially reversed these alterations by modulating genes involved in adipocyte differentiation, insulin sensitivity, lipid metabolism, and thermogenesis, including Pparg, Adipoq, Slc2a4, Lpl, Ucp1, and Dio. Linagliptin also reshaped the miRNA profile, partially restoring HF-altered miRNAs and regulating additional miRNAs associated with inflammatory and metabolic pathways. Bioinformatic analyses supported coordinated regulatory networks linked to adipogenesis, oxidative metabolism, and inflammatory signaling. In silico analysis identified Twist1 as a putative target of miR-325-3p, suggesting a potential post-transcriptional mechanism involved in adipose tissue remodeling.

CONCLUSIONI

Overall, linagliptin induced coordinated transcriptional and post-transcriptional remodeling of VAT. Among the pathways analyzed, no molecular mechanisms specifically promoting weight loss or opposing fat mass reduction emerged, consistent with the weight-neutral profile of DPP-4 inhibition. The miR-325-3p/Twist1 axis may contribute to adipose tissue remodeling, while additional uninvestigated pathways may account for the dissociation between metabolic improvement and adipose mass reduction.

A012

MODELLING FUNCTIONAL AND METABOLIC ALTERATIONS IN AN EXPERIMENTAL HUMAN-DERIVED MODEL OF STEATOTIC-LIKE HEPG2 HEPATOCYTES BY EXPLOITING A NOVEL SUSTAINABLE EXTRACT OF MALUS DOMESTICA CRIPPS PINK APPLE EXTRACT

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INTRODUZIONE

Metabolic dysfunction-associated steatotic liver disease (MASLD) is among the most prevalent chronic liver diseases worldwide and is characterized by lipid accumulation, oxidative stress, and metabolic dysfunction in hepatocytes. Reliable human-derived experimental models are needed to investigate disease mechanisms and identify novel therapeutic strategies. Natural bioactive compounds from sustainable sources are emerging as promising tools for metabolic disease management, from prevention to treatment, also in combination (add-on therapy) with pharmacological therapy.
This study aimed to establish a steatotic-like HepG2 model and evaluate the protective effects of a novel and sustainable Malus domestica Cripps Pink apple extract on steatosis-associated metabolic alterations.

METODI

Human hepatoma HepG2 cell line and the steatosis-like model of HepG2 cells induced with oleic acid (HepG2-OA) were used. Following steatosis assessment in HepG2-OA, both models were treated with a sustainable Malus domestica Cripps Pink apple extract (0.01, 0.1, 0.5, 1 mg/mL) for 6/24/48 h. After treatment, cell viability (MTT assay), glucose uptake (6-NBDG)/oxidative stress (flow cytometry), lipid droplet accumulation (Oil Red O, ORO staining) and gene expression (RT-qPCR) were evaluated in both models.

RISULTATI

Malus domestica Cripps Pink apple extract showed no cytotoxicity in either model after 24/48 h treatment. The extract increased glucose uptake (****$p<0.0001$) and reduced ROS production (***$p<0.001$), together with a dose-dependent downregulation of SOD and CAT expression. Moreover, treatment significantly decreased intracellular lipid droplet accumulation (****$p<0.0001$) and reduced DGAT1 and FASN expression in a dose-dependent manner in both HepG2 and HepG2-OA cells.

CONCLUSIONI

The proposed steatotic-like HepG2 model successfully reproduces key metabolic features of hepatic steatosis. Treatment with sustainable Malus domestica Cripps Pink apple extract improved glucose metabolism, reduced oxidative stress, and attenuated lipid accumulation, supporting its potential as a promising strategy for the prevention and management of MASLD-associated metabolic alterations.

A013

RESVERATROL AND ITS ANALOGUE WITH SIMILAR METABOLIC PROFILES SYNERGISTICALLY ENHANCE CISPLATIN CYTOTOXICITY, INDUCING DNA DAMAGE IN A549 LUNG CANCER CELLS

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INTRODUZIONE

The treatment of lung cancer is heavily challenged by drug resistance and the toxicity of standard chemotherapeutic agents like cisplatin (CDDP). To overcome these limitations, combination therapies with naturally derived molecules has gained significant relevance. Among these, resveratrol (RSV), a well-known stilbene, has already shown synergistic effects with CDDP in vitro. However, less is known about its structural analogue, 4,4'-dihydroxy-trans-stilbene (DHS), which exhibits superior antiproliferative and antioxidant activities at lower concentrations.
This study investigate the combined effects of RSV and DHS with cisplatin on the A549 non-small cell lung cancer (NSCLC) cell line.

METODI

Using MTT assays and mathematical synergy models, the combinatorial landscapes of stilbene-CDDP mixtures were obtained at various concentrations to quantify their impact on A549 cell viability at 48-72 hours. DNA damage was evaluated both directly, with the COMET assay; and indirectly, with a bi-parametric PI and γH2AX staining using flow cytometry. Moreover, a preliminary investigation using an Ultra-High Performance Liquid Chromatography - Mass Spectrometry (UHPLC-MS) system of the hypothesised metabolites of DHS, was conducted based on masses correlations, on both cell pellets and supernatants.

RISULTATI

These results revealed a high synergistic landscape for both combinations, which was more broadly defined for DHS +CDDP, according to the models adopted. Flow cytometry analysis highlighted cell cycle alterations and a marked increase in γH2AX positivity in the pre-G1 phase, indicative of cell death, confirming the higher efficacy of the DHS +CDDP in inhibiting cell viability. From the UHPLC-MS analysis it was observed the presence of the hypothesised DHS metabolites especially in the supernatants, showing a good correlation with their theoretical masses.

CONCLUSIONI

These data suggest that both stilbenes, especially DHS, are able to synergically reduce cell viability at 48-72 hours on A549, inducing DNA damage through distinct molecular pathways, when combined with CDDP. Furthermore, the preliminary investigation of the hypothesised DHS metabolites suggest their presence, indicating a similar metabolic outcome to RSV.

A014

ANTI-BETA 4 INTEGRIN ANTIBODIES IN PATIENTS WITH MUCOUS MEMBRANE PEMPHIGOID: AN EUROPEAN MULTICENTRIC STUDY

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INTRODUZIONE

Mucous Membrane Pemphigoid (MMP) is a rare and heterogeneous group of autoimmune blistering diseases mostly affecting mucosal surfaces and characterized by autoantibodies against different basement membrane zone (BMZ) components. Diagnosis mainly relies on invasive immunofluorescence-based techniques, which may be difficult to perform depending on the anatomical site involved. Among the less investigated autoantigens, β4 integrin has emerged as a potential diagnostic biomarker. This study aimed to evaluate the frequency and diagnostic relevance of anti-β4 integrin autoantibodies in a large cohort of MMP patients from different geographic areas.

METODI

Sera from MMP patients were analyzed for anti-β4 integrin autoantibodies by immunoblotting (IB) using lysates from the A431 cell line. Healthy donors were included as negative controls, while sera from patients with bullous pemphigoid (BP) were used as disease controls. Assay specificity was validated using β4 integrin-silenced A431 cells.

RISULTATI

A total of 96 sera were analyzed: 69 from MMP patients (collected in Florence, Rome, and Germany), 11 from BP patients, and 16 from healthy donors. Positivity was defined by the presence of the band at 250 kDa. Anti-β4 integrin reactivity was detected in 17/69 MMP patients (24,6%) and in 1/11 BP patients (9,1%), while healthy donors showed no reactivity.

CONCLUSIONI

Our findings demonstrate a high frequency of anti-β4 integrin autoantibodies in MMP sera, supporting their potential role as an additional diagnostic biomarker. Their detection may contribute to a more accurate and less invasive diagnosis of MMP, particularly in cases lacking reactivity against more common autoantigens. Further investigation in larger, multicenter cohorts is warranted to validate these findings.

A015

PROTEOMICS OF PERI-PANCREATIC ADIPOSE TISSUE IN OVERWEIGHT/OBESITY AND IN TYPE 2 DIABETES

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INTRODUZIONE

Visceral adipose tissue (AT) is associated with type 2 diabetes (T2D). To date, no information is available on the molecular features of peri-pancreatic AT (PAT) in human T2D. We analyzed proteomics of PAT from non-diabetic (ND) and T2D subjects and the impact of overweight/obesity (OW/OB).

METODI

PAT was obtained from 52 organ donors, whose characteristics (median [IQR]) were: 15 normal weight (NW-ND): (M/F: 11/4; age: 75 [64-86] years; BMI: 23.7 [22.0-24.5] kg/m^2), 8 OW/OB-ND (M/F: 3/5; age:66 [56-70] years; BMI: 271 [25.9-28.1] kg/m^2], 8 NW-T2D (M/F: 2/6; age:81 [75-83] years; BMI:23.1 [22.6-23.4] kg/m^2) and 21 OW/OB-T2D (M/F: 15/6; age:76 [66-79] years; BMI:27.6 (26.0-28.9) kg/m^2). Peptides were analyzed by LC-MS/MS. Protein abundance in OW/OB-ND, NW-T2D, and OW/OB-T2D groups was compared with NW-ND group.

RISULTATI

Overall, 4,827 proteins were identified, and 1,595 were differentially expressed (ANOVA $p \leq 0.01$). Analyses revealed 100 proteins up-regulated (UP) in OW/OB-ND (36 in common with NW-T2D and 24 with OW/OB-T2D); 163 UP in NW-T2D (68 in common with OW/OB-T2D), and 182 UP in OW/OB-T2D. The top 5 specific for each group with the highest abundances included: STX5, RNF6, GUK1, NDUFB9 and ANK1 in OW/OB-ND; DUSP3, H3C1, HSP90AB3P, MYO5C and TCEAL3 in NW-T2D; RAP1B, DHRS1, PCNA, LPIN1 and SETD7 in OW/OB-T2D. The same analysis revealed 157 proteins down-regulated (DR) in OW/OB-ND (53 in common with NW-T2D and 35 with OW/OB-T2D); 126 DR in NW-T2D (42 in common with OW/OB-T2D), and 111 DR in OW/OB-T2D. The top 5 specific for each group showing the lowest abundances included: CLIC6, CRLF1, HNRNPCL2, SH3BGRL and SLC1A5 in OW/OB-ND; ACBD5, ARMC10, DHRS1, SRSF9 and THRSP in NW-T2D; BSDC1, COMMD1, LSM7, LHPP and SLC25A10 in OW/OB-T2D.

CONCLUSIONI

In conclusion, proteomics of PAT showed several abundance differences associated with BMI values and glycemia status. These data contribute to the understanding of the role of PAT in OW/OB and T2D.

A016

S-ADENOSYLMETHIONINE ENHANCES TEMOZOLOMIDE SENSITIVITY AND OVERCOMES MGMT-MEDIATED RESISTANCE IN GLIOBLASTOMA CELLS

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INTRODUZIONE

Glioblastoma multiforme (GB) is the most aggressive primary brain tumor, characterized by rapid progression, high recurrence rates, and poor prognosis. Although temozolomide (TMZ) remains the standard chemotherapeutic agent for GB, the onset of drug resistance, mainly associated with MGMT activity and tumor genetic plasticity, severely limits treatment efficacy. S-Adenosylmethionine (AdoMet), a natural methyl donor with antiproliferative and epigenetic regulatory properties, has recently emerged as a potential adjuvant in cancer therapy. This study investigated the ability of AdoMet to enhance TMZ sensitivity and overcome resistance mechanisms in GB cells.

METODI

The effects of AdoMet and TMZ, administered alone or in combination, were evaluated in TMZ-resistant T98G GB cells and patient-derived GB cultures with unmethylated MGMT promoter status. Drug interactions were analyzed using the Chou-Talalay method to determine Combination Index (CI) and Dose Reduction Index values (DRI). Cell proliferation and confluence were monitored by digital holographic microscopy, while apoptosis was assessed by FACS and Western blotting analysis. MGMT expression was analyzed by qRT-PCR and Western blotting.

RISULTATI

AdoMet strongly potentiated TMZ antitumor activity, resulted in strong synergistic interactions, as indicated by CI values well below 1. Combination treatment significantly reduced the effective TMZ dose, with DRI values exceeding 5-fold at lower fractions affected. Digital holographic microscopy demonstrated a pronounced reduction in cell proliferation and confluence following combined treatment compared with single drugs. Moreover, co-treatment significantly enhanced apoptosis, reaching approximately 35% apoptotic cells, and markedly increased PARP-1 cleavage. Importantly, AdoMet significantly reduced MGMT expression at both mRNA and protein levels in T98G and patient-derived GB cells, sensitizing the cells to TMZ effects and suggesting a cooperative mechanism to overcome TMZ resistance.

CONCLUSIONI

Our data demonstrate that AdoMet enhances TMZ efficacy by suppressing MGMT-mediated resistance. AdoMet may therefore represent a promising natural adjuvant strategy to improve the therapeutic response in TMZ-resistant GB.

A017

METTL16-MEDIATED INHIBITION OF MXD4 PROMOTES ACUTE MYELOID LEUKEMIA THROUGH ACTIVATION OF MYC-MAX AXIS

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INTRODUZIONE

N6-methyladenosine (m^6A) governs multiple facets of RNA metabolism, including splicing, translation, stability, decay, and the processing of marked transcripts. Accumulating evidence has suggested that the m6A writer METTL16 is involved in leukemia, however, the molecular pathways by which it contributes to leukemogenesis remain unexplored.

METODI

Here we use a shRNA approach to characterize the role of METTL16 in acute myeloid leukemia (AML) proliferation in vitro and in ovo. We evaluated the phenotypic effect of METTL16 knockdown (KD) and determined transcriptomic and epi transcriptomic perturbations in shMETTL16 cells by RNA-seq and meRIP-seq analysis. We confirmed METTL16 regulation of MXD4 through RNA immunoprecipitation (RIP), Actinomycin D, Western Blot (WB), and co-immunoprecipitation (Co-IP) analysis. Lastly, concomitant KD of METTL16 and MXD4 rescued the leukemic phenotype

RISULTATI

Our investigation revealed that METTL16 is overexpressed in AML samples compared to the normal counterpart. Genetic depletion of METTL16 strongly affected the growth and metabolic rates of AML cells, eventually triggering apoptosis. Transcriptome-wide analysis identified MXD4 mRNA, an MYC-pathway regulator, as a downstream target of METTL16. Mechanistically, we demonstrate that METTL16 controls the stability of MXD4 mRNA resulting in the reduction of MDX4 protein levels that indirectly activate the MYC-MAX axis, which is essential for leukemogenesis. Strikingly, suppression of MXD4 rescued the expression levels of MYC genes restoring AML cell growth.

CONCLUSIONI

Our findings disclose a novel METTL16-MXD4 oncogenic axis crucial for AML survival, advancing METTL16 as a novel therapeutic target in AML and providing a new strategy to target MYC activity in cancer.

A018

THE POTENTIAL ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF STRAWBERRY EXTRACT SABRINA ON LPS-INDUCED INFLAMMATION IN THP-1-DERIVED MACROPHAGES

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INTRODUZIONE

Strawberries are well known as a rich source of bioactive phytochemicals and antioxidants associated with potential protective effects against several chronic diseases. In this context, the present study focuses on the Sabrina cultivar, a modern breeding-derived variety developed for improved agronomic performance, including stress tolerance and higher productivity. Beyond its commercial traits, this work investigates the potential nutraceutical properties of Sabrina strawberry extracts, specifically evaluating their antioxidant and anti-inflammatory activities in THP-1derived macrophages.

METODI

Cytotoxicity of Sabrina strawberry extract was evaluated in THP-1 cells by assessing cell metabolic activity. THP-1derived macrophages were treated with increasing concentrations of the extract and subsequently stimulated with LPS to investigate modulation of inflammatory responses. Intracellular reactive oxygen species (ROS) production was quantified to evaluate oxidative stress levels. Expression of key proteins and genes involved in the NF-κB signaling pathway was then analyzed by Western blot and qRT-PCR.

RISULTATI

Cell viability in THP-1-derived macrophages was not affected by Sabrina extract up to 200 μg/mL, indicating no cytotoxic effects. Sabrina extract significantly reduced LPS-induced intracellular ROS production, indicating a marked attenuation of oxidative stress. Consistently, qRT-PCR analysis showed a significant decrease in the LPS-induced expression of pro-inflammatory cytokines, including IL-6 and IL-8. In addition, Western blot analysis revealed inhibition of LPS-induced activation of the NF-κB signaling pathway.

CONCLUSIONI

In conclusion, Sabrina strawberry extract exerts significant antioxidant and anti-inflammatory effects in LPSstimulated THP-1-derived macrophages. These findings support the potential of the Sabrina cultivar as a promising source of bioactive compounds with possible nutraceutical applications.

A019

DUAL TARGETING OF PI3K AND AURORA B PATHWAYS PRODUCED AN ANTI-TUMOR RESPONSE IN PRECLINICAL MODELS OF NSCLC HARBORING PIK3CA MUTATIONS.

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INTRODUZIONE

Mutations in the p110α subunit of class I phosphatidylinositol 3-kinase, encoded by the PIK3CA gene, are detected in approximately 4–6% of patients with lung adenocarcinoma (LUAD). These alterations predominantly occur in the helical domain (E545K in exon 9) or in the kinase domain (H1047R in exon 20) and are widely recognized as oncogenic and potentially targetable. However, despite their well-established oncogenic role, no approved therapies specifically targeting PIK3CA mutations in LUAD are currently available. This project aims to elucidate the contribution of these mutations to the activation of alternative signaling pathways and to identify novel targeted therapeutic strategies.

METODI

H322 and H292 cells were transfected with plasmids encoding PIK3CA H1047R or E545K mutations. Cell proliferation, colony assay, and migration were monitored using the HoloMonitor system, crystal violet staining, and transwell migration assay. A mid-throughput drug screening was performed using a panel of 176 compounds to identify agents synergizing with a PI3K inhibitor. Intracellular signaling pathways were analyzed by Western blotting. Drug combination effects were quantified using Combenefit software. Apoptosis was assessed by Annexin V staining.

RISULTATI

Increased migration, 2D and 3D growth rate were observed in cells harboring PIK3CA mutations, accompanied by increased PI3K/Akt/mTOR pathway activity and the acquisition of a mesenchymal phenotype. The drug screening approach identified the Aurora kinase A/B inhibitor AT9283 as the most promising compound for combination with the PI3K inhibitor CH5132799. Combined treatment of CH5132799 and AT9283 reduced cell proliferation, colony formation, and cell migration in both cells harbouring H1047R and E545K mutations and induced cell apoptosis. Clones exhibited high levels of only Aurora-B phosphorylation, and the combination of the specific Aurora kinase B inhibitor (AZD1152) with CH5132799 promoted a synergistic antiproliferative effect both in 2D and 3D systems compared to parental cells.

CONCLUSIONI

Our findings suggest that co-targeting PI3K and Aurora kinase B may represent a novel therapeutic approach for NSCLC patients with PIK3CA mutations, with the potential to improve clinical outcomes.

A020

IL-33-ASSOCIATED INFLAMMATORY SIGNALING AND PPAR-DRIVEN METABOLIC REPROGRAMMING IN HIGH-FAT DIET–INDUCED CARDIAC PROTEOME REMODELING

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INTRODUZIONE

High-fat diet (HFD) is a major contributor to obesity, metabolic syndrome, and cardiovascular disease. Excess lipid availability promotes cardiac metabolic inflexibility, mitochondrial dysfunction, and lipotoxic damage. Emerging evidence suggests that inflammatory mediators, including the IL-33 signaling axis, may contribute to the interplay between metabolic stress and cardiac remodeling. However, the proteomic mechanisms linking HFD-induced metabolic dysregulation to inflammatory and structural cardiac alterations remain incompletely understood.
To characterize HFD-induced proteomic remodeling in cardiac tissue and identify the molecular pathways involved in metabolic and inflammatory cardiotoxicity.

METODI

Cardiac tissues from high-fat diet (FH) and low-fat diet (LH) groups were analyzed using a label-free LC–MS/MS proteomic approach. Data were processed using MaxQuant and Perseus. Differentially expressed proteins were subjected to functional enrichment analyses using Panther, DAVID, ClueGO, STRING, and Ingenuity Pathway Analysis (IPA). Integrated proteomic–transcriptomic analyses were also performed.

RISULTATI

A total of 1381 proteins were identified in the FH group and 1265 in the LH group, with 340 proteins significantly upregulated and 254 downregulated in FH samples ($p \leq 0.05$). Integrated analyses revealed marked activation of mitochondrial fatty acid β-oxidation pathways and lipid metabolic processes, associated with reduced oxidative phosphorylation and enhanced sirtuin signaling. Upstream regulator analysis identified PPARα activation and MYC inhibition as central drivers of metabolic remodeling. Notably, IL-33–associated inflammatory signaling emerged as a key regulatory axis linking metabolic stress to cardiac inflammatory responses. In addition, Caveolin-1 was identified as a potential mediator connecting PPAR signaling, endothelial dysfunction, and lipotoxic remodeling.

CONCLUSIONI

HFD induces profound cardiac proteomic remodeling characterized by PPAR-dependent metabolic reprogramming, mitochondrial dysfunction, and IL-33–associated inflammatory activation. These findings provide novel insights into the molecular mechanisms underlying metabolic cardiomyopathy and highlight potential therapeutic targets at the intersection of metabolic and inflammatory signaling.

A021

DECODING THE EPIGENETIC LANDSCAPE SHAPED BY OBESITY-DERIVED EXTRACELLULAR VESICLES IN MAMMARY EPITHELIAL CELLS: UBE2C AS A POTENTIAL DRIVER OF EARLY BREAST ONCOGENESIS

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INTRODUZIONE

Obesity represents one of the most important modifiable risk factor in breast cancer (BC). This pathological condition is characterized by systemic metabolic and inflammatory alterations that reshape the secretome of adipose tissue, including the quantity and composition of extracellular vesicles (EVs). EVs are emerging as key mediators of intercellular communication, capable of transferring bioactive cargo and reshaping regulatory and epigenetic programs in recipient cells. However, the mechanisms through which obesity-derived EVs induce early epigenetic and oncogenic transformation of mammary epithelial cells remain poorly understood.

METODI

As experimental models we used MCF-10A cells chronically exposed to EVs isolated from the serum of healthy women stratified by body mass index (BMI) into normal weight (NW) and overweight/obese (OW/Ob) groups, generating NW-EV- and OW/Ob-EV-educated cells. Circulating EVs were isolated and characterized according to the MISEV23 guidelines. Phenotypic alterations induced by EV exposure were assessed through proliferation, migration, and metabolic assays, as well as by qRTPCR and transcriptomic analyses.

RISULTATI

OW/Ob-educated MCF-10A cells displayed a more aggressive phenotype compared to NW-educated cells, characterized by increased growth, motility, invasion, expression of mesenchymal markers and by altered mitochondrial activity. RNA-seq analysis identified 22 genes significantly deregulated between OW/Ob-EV- and NW-EV-educated MCF-10A cells. Ubiquitin-Conjugating Enzyme E2C (UBE2C) emerged as the most significantly upregulated gene and a central hub within the network. Interestingly, these cells also displayed enhanced sensitivity to the epigenetic drug Vorinostat, a known modulator of UBE2C-related pathways.

CONCLUSIONI

Our data show that obesity-derived EVs promote oncogenic transformation of mammary epithelial cells and suggest that this process may involve epigenetic reprogramming associated with UBE2C expression. Given the rising prevalence of obesity, elucidating the mechanisms through which circulating OW/Ob-EVs drive epigenetic remodeling and malignant transformation of breast epithelial cells may provide more effective strategies for the clinical management of this high-risk population.

A022

THE EXPRESSION OF THE P62/SQSTM1 PROTEIN IS REGULATED BY THE LYSINE-METHYLTRANSFERASE KMT5A AND THE P53 PROTEIN IN GLIOBLASTOMA

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INTRODUZIONE

KMT5a is the sole histone lysine methyltransferase responsible for histone H4 lysine 20 monomethylation (H4K20me1). KMT5a is overexpressed in various cancers, including glioblastoma.
It has been shown that KMT5a-induced H4K20me1 can regulate the expression of various genes. However, its role in transcription is still not fully understood.
Therefore, we performed RNA sequencing and found that expression of the key autophagy adaptor p62/SQSTM1 was strongly increased following KMT5a inhibition.

METODI

To investigate how KMT5a could regulate p62/SQSTM1 expression, impairment of KMT5a was performed in U251 glioblastoma cells. Chromatin immunoprecipitation (ChIP) assays assessed KMT5a binding to the p62/SQSTM1 promoter and histone modification status.
The role of the additional methyltransferases KMT5b and KMT5c, which catalyze H4K20me2 and H4K20me3, respectively, was also assessed.
We also evaluated the relationship between p53 status and p62/SQSTM1 expression using various glioblastoma cell lines with different p53 backgrounds, as well as through genetic manipulation of p53 expression.
In addition, we examined this dependency in primary glioblastoma cells and glioblastoma stem cells.

RISULTATI

Inhibition or knockdown of KMT5a led to a strong increase in p62/SQSTM1 transcription in U251 glioblastoma cells. ChIP assays confirmed direct binding of KMT5a to the p62/SQSTM1 promoter and deposition of the H4K20me1 mark. We found that the inhibition of H4K20me1/2/3 lead to a higher induction of p62/SQSTM1, demonstrating that full repression indicated a multi-step repression mechanism.
Moreover, p62/SQSTM1 induction was observed only in p53-deficient cells and was suppressed by reintroduction of wild-type p53. Conversely, expression of dominant-negative p53 pcDNA in p53-proficient cells restored p62/SQSTM1 induction.
We also observed that starvation-induced expression of p62/SQSTM1 also required both H4K20me1 demethylation and p53 displacement from its promoter.

CONCLUSIONI

We demonstrated that KMT5a is an epigenetic repressor of p62/SQSTM1 in glioblastoma cells, through a mechanism dependent on histone methylation and p53. In a future perspective, these findings also suggest that KMT5a inhibition could serve as a therapeutic strategy specifically in p53-proficient glioblastomas, where p62/SQSTM1 remains repressed.

A023

HELICOBACTER PYLORI INDUCES THE PRODUCTION OF INTERLEUKIN-37 FROM HUMAN GASTRIC MUCOSA TO PROMOTE IMMUNOSUPPRESSION, ESCAPING SPECIFIC T CELL RESPONSE.

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INTRODUZIONE

Helicobacter pylori (HP) infects the gastric mucosa of approximately half of the world's population, residing within the host for life by modulating human immunity through various known and unknown mechanisms, thereby causing a spectrum of diseases ranging from gastritis to gastric ulcers and cancer. In our study, we aimed to examine the role of Interleukin (IL)-37 on host immune response and on HP persistence in the stomach.

METODI

Firstly, the presence of IL-37 within the gastric mucosa was determined by immunohistochemistry and qRT-PCR analysis of biopsy specimens collected from nine HP-positive individuals and nine healthy controls. Subsequently, to investigate in vitro the role of HP in inducing IL-37 production by gastric epithelial cells, we quantified by Luminex assay the IL-37 secretion by gastric mucosoids derived from the stomach of three healthy subjects. Results obtained by HP co-cultured mucosoids versus uninfected ones were compared. Finally, HP-specific T lymphocytes, infiltrating the stomach of ten HP-infected patients, were isolated and tested for their proliferating ability in response to HP lysate, with or without adding rIL-37 in their culture medium.

RISULTATI

IL-37 was significantly increased in the gastric mucosa of HP-infected individuals compared to non-infected controls ($P = 0.0056$). Higher levels of secreted IL-37 were found in HP-infected mucosoids compared to uninfected ones ($P < 0.0001$). A dose-dependent decrease in the proliferation index of HP-specific T cells stimulated with bacterial lysate was observed with increasing rIL-37 concentrations (0.1, 1, and 10 ng) in the culture medium ($P < 0.0001$).

CONCLUSIONI

The study demonstrates that HP induces an increase in the production of IL-37 in the human gastric mucosa as a mechanism to evade the host immune response. Specifically, IL-37 modulates human HP-specific T cell functions in a dose-dependent manner, promoting immunosuppression to facilitate bacterial persistence in the host.

A024

INTERLEUKIN 8 AS A POTENTIAL MEDIATOR OF OBESITY-ASSOCIATED INFLAMMATION IN MULTIPLE MYELOMA PROGRESSION

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INTRODUZIONE

Obesity is increasingly recognised as a factor influencing incidence and clinical progression of multiple myeloma (MM), a plasma cell malignancy accounting for 10–15% of all haematological cancers. Obese patients are characterized by low-grade inflammation and altered metabolic markers which might influence MM progression and aggressiveness. Although the molecular mechanisms linking obesity to MM remain poorly understood. The aim of this study was to evaluate the impact of the serum from healthy donors, classified by body mass index (BMI), on modulating the MM cell phenotype.

METODI

RPMI-8226 and U-266 MM cell lines were cultured with pooled sera from normal weight (NW; n=20, BMI≤24.9 kg/m²) or OW/Ob (n=20, BMI≥25 kg/m²) healthy donors. Cell viability, proliferation, migration, invasion and colony formation assays were performed. Serum cytokine profiles were analysed by multiplex immunoassays. Protein interaction networks was explored by STRING analysis. OW/Ob sera were depleted of selected inflammatory cytokine and depletion efficiency was confirmed by ELISA. Depleted-sera were then used to evaluate the role of identified inflammatory mediator in biological assays.

RISULTATI

OW/Ob sera promoted a more aggressive MM phenotype, increasing colony formation, migration and invasion, compared to NW sera. Cytokine profiling revealed enrichment of pro-inflammatory and pro-tumorigenic mediators together with reduced immune-modulatory factors in OW/Ob sera. STRING analysis identified IL-8 (CXCL8) as a central hub within the obesity-associated inflammatory network. IL-8 immunodepletion from OW/Ob sera significantly reduced MM cell motility and clonogenic potential promoted by OW/Ob complete sera, supporting a direct role for IL-8 in obesity-driven MM aggressiveness.

CONCLUSIONI

OW/Ob sera promote MM cell aggressiveness through an altered inflammatory cytokine profile, with IL-8 emerging as a key mediator linking obesity-associated inflammation to MM progression. IL-8 depletion reduced aggressive MM features, supporting IL-8 targeting as a potential strategy to modulate the tumour-supportive obesity-milieu in MM.

A025

DNA METHYLATION PROFILING IDENTIFIES EPIGENETIC REPROGRAMMING IN FULVESTRANT RESISTANT ER+ BREAST CANCER

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INTRODUZIONE

Endocrine therapy is a standard treatment for estrogen receptor–positive (ER+) breast cancer. Fulvestrant, a selective estrogen receptor degrader, is widely used in this setting. However, both primary and acquired resistance limit its clinical benefit. Although several mechanisms of endocrine resistance have been described, the molecular basis of fulvestrant resistance and the role of DNA methylation remain unclear. Therefore, we investigated whether DNA methylation changes are involved in fulvestrant resistance.

METODI

We performed integrative methylome and transcriptome analysis in five paired fulvestrant-sensitive and -resistant breast cancer cell line models using Infinium HD Methylation assay and RNA sequencing.

RISULTATI

Resistant cell lines showed higher global DNA methylation than matched sensitive counterparts. Consistently, low-dose DNA methyltransferase inhibitor decitabine had a stronger effect ($p<0.001$) in resistant cells. Pairwise analysis of shared differentially methylated CpGs revealed enrichment at ESR1 binding sites, particularly within hypermethylated regions. Hypermethylated CpGs were mainly located at transcription factor binding sites involved in hormone signaling and luminal identity. In contrast, hypomethylated CpGs were enriched in regulatory regions associated with progenitor/stem-like programs, as well as chromatin remodeling. By integrating DNA methylation at promoter and enhancer regions with gene expression data, we identified genes with significant inverse methylation–expression correlations. Hypermethylation with transcriptional downregulation affected genes linked to luminal differentiation, growth control, cell adhesion, endocrine response, and tumor suppression. Conversely, hypomethylation with transcriptional upregulation characterized genes involved in interferon signaling, metabolic adaptation, developmental pathways, and hypoxia.

CONCLUSIONI

These findings reveal epigenetic reprogramming associated with fulvestrant resistance in breast cancer, characterized by altered DNA methylation at regulatory regions linked to luminal identity, endocrine response, and adaptive/stem-like programs. Overall, these results highlight DNA methylation changes as potential biomarkers and therapeutic vulnerabilities in fulvestrant-resistant breast cancer.

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A026

NEUROPSYCHIATRIC DISORDERS DURING TREATMENT FOR ADHD WITH ATOMOXETINE AND METHYLPHENIDATE A SYSTEMATIC REVIEW AND META-ANALYSIS

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INTRODUZIONE

Although atomoxetine (ATX) and methylphenidate (MPH) are widely prescribed and effective treatments for attention-deficit/hyperactivity disorder (ADHD), concerns about potential neuropsychiatric adverse events continue to affect prescribing decisions and adherence to treatment. This study aimed to assess the risk of neuropsychiatric adverse events associated with ATX and MPH in patients with ADHD, based on evidence from RCTs.

METODI

A systematic literature search was conducted in MEDLINE (PubMed), Embase and Web of Science between 1 January 2007 and 19 February 2026. RCTs evaluating ATX or MPH in patients diagnosed with ADHD were included. Eligible studies comprised placebo-controlled trials and direct comparisons between ATX and MPH. Risk ratios (RRs) and their 90% confidence intervals (CIs) were pooled using a random-effects model for both placebo-controlled and head-to-head comparisons. The primary outcome was the occurrence of neuropsychiatric AEs, with additional stratified analyses by age.

RISULTATI

A total of 54 RCTs were included. Compared with a placebo, ATX was associated with significantly increased risks of irritability ((RR),1.59; 90%CI, 1.07–2.36) and insomnia (RR, 2.16; 90% CI, 1.53–3.05). MPH was associated with a higher risk of anxiety (RR: 4.15; 90%CI: 2.55–6.76), initial insomnia (RR: 3.02; 90% CI: 1.87–4.89), insomnia (RR: 2.34; 90%CI: 1.72–3.18) and restlessness (RR: 2.04; 90%CI: 1.12–3.70). Analyses of disproportionality for suicidality did not identify any statistically significant safety concerns for either drug. In head-to-head comparisons, no statistically significant differences in neuropsychiatric AEs were observed between treatments. Age-stratified analyses revealed a higher risk of insomnia associated with methylphenidate use among younger patients (RR: 2.37; 90%CI: 1.22–4.62) than among adults (RR: 1.72; 90%CI: 1.33–2.23).

CONCLUSIONI

Overall, these findings suggest that neuropsychiatric AEs may be a class-related risk rather than a drug-specific effect, given the available randomized evidence. Further evidence will require integration with observational meta-analyses.

A027

PHARMACOLOGICAL INDUCTION OF A REVERSIBLE DORMANCY-LIKE STATE BY THE GLUCOCORTICOID BUDESONIDE RESTRAINS TUMOR GROWTH IN COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality due to intratumoral heterogeneity and cellular plasticity, which promote therapy resistance and disease relapse. Patient-derived organoids (PDOs) represent a robust platform to investigate dynamic cancer cell states, including cellular dormancy. Here, we evaluated the clinically approved glucocorticoid budesonide as a modulator of CRC cell plasticity and dormancy.

METODI

CRC PDOs and matched xenograft models were treated with budesonide to evaluate its effect on tumor growth dynamics and cellular plasticity. Integrated morphological, transcriptional, and functional analyses were used to characterize metabolic adaptation, stemness-associated programs, and dormancy-related features.

RISULTATI

Budesonide significantly impaired PDO growth and lumen maturation in a dose- and time-dependent manner without affecting cell viability or clonogenic potential. Consistently, budesonide treatment reduced tumor growth in CRC xenograft models. Transcriptomic and functional analyses revealed coordinated downregulation of protein synthesis, oxidative metabolism, and proteasome activity, together with activation of dormancy-associated programs and enrichment of intestinal stem cell signatures. Budesonide treatment increased the proportion of slow-cycling label-retaining cells while preserving long-term self-renewal capacity. Notably, growth arrest was fully reversible after drug withdrawal, consistent with a dormant-like phenotype. Mechanistically, these effects were, at least in part, independent of canonical glucocorticoid receptor signaling.

CONCLUSIONI

Our findings identify budesonide as a pharmacological inducer of a reversible dormant-like state in CRC models. These results highlight cellular dormancy as a drug-modulable program with potential translation relevance for limiting tumor progression and therapeutic resistance.

A028

HUR AS A THERAPEUTIC VULNERABILITY IN CASTRATION-RESISTANT PROSTATE CANCER

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INTRODUZIONE

Prostate cancer (PC) is one of the most frequently diagnosed cancers in men and is commonly considered a "hormone-dependent cancer". PC is initially an androgen-dependent disease and at this stage, the androgen deprivation therapy (ADT) represents the first-line therapeutic option. Although initially effective, this therapy frequently fails and the disease progresses to an androgen-resistant state, also known as castration-resistant PC (CRPC), commonly characterized by poor prognosis. New efforts are needed to unravel the mechanism(s) underlying PC hormone-resistance and spreading. HuR (ELAVL1), an RNA-binding protein that regulates mRNA stability and translation of genes involved in proliferation, survival and stress responses, is implicated in PC progression and prognosis. These findings make it as a clinically actionable target in CRPC.

METODI

To investigate HuR (ELAVL1) expression and functional activity, clinical and transcriptomic data from the SU2C/PCF metastatic CRPC cohort were analyzed. Survival and group comparisons were performed utilizing Kaplan-Meier estimates, log-rank, and Wilcoxon rank-sum tests. HuR pharmacological inhibition in various CRPC-derived cell lines was assessed through DNA synthesis, colony formation, and 3D culture analyses.

RISULTATI

HuR transcript levels showed no significant differences between antiandrogen-naive and -resistant patients, indicating that therapy resistance is not driven by HuR transcriptional upregulation. Conversely, an elevated HuR target signature score significantly correlated with worse overall survival, emphasizing the clinical impact of HuR-mediated oncogenic programs. In vitro, treatment with novel HuR inhibitors reduced the proliferation rate in all the CRPC-derived cell lines analysed and impaired their long-term clonogenic and 3D growth.

CONCLUSIONI

HuR functional activity, rather than its expression alone, correlates with poor clinical outcome in CRPC. Pharmacological inhibition using newly developed HuR inhibitors suppresses proliferation, clonogenic potential and 3D growth across various CRPC models, supporting the hypothesis that HuR represents a clinically actionable target in advanced disease.

A029

TARGETING TRPM8 IN METASTATIC MELANOMA: A DUAL STRATEGY FOR APOPTOSIS INDUCTION AND NK CELL SENSITIZATION

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INTRODUZIONE

Metastatic melanoma remains a major clinical challenge due to therapeutic resistance and immune evasion. The transient receptor potential melastatin 8 (TRPM8) ion channel is overexpressed in several malignancies, altough its role in melanoma progression and immune regulation is still poorly understood. We investigated the anti-tumor and immunomodulatory effects of novel TRPM8 modulators in metastatic melanoma model.

METODI

TRPM8 expression was evaluated in metastatic melanoma cell lines (AMM16, WM266-4) and normal skin cells. The anti-tumor activity of two novel TRPM8 antagonists (compounds 4 and 9) was assessed in both 2D cultures and 3D spheroids. Cell death mechanisms were analyzed through viability assays, ROS quantification, mitochondrial membrane potential analysis and apoptotic marker evaluation. Immunomodulatory effects were explored using ATM/p53 silencing and co-culture assays with primary Natural Killer (NK) cells.

RISULTATI

TRPM8 was markedly overexpressed in metastatic melanoma cells compared with normal counterparts. Pharmacological inhibition of TRPM8 selectively induced calcium-independent mitochondrial apoptosis, characterized by ROS accumulation, mitochondrial depolarization, cytochrome c release, and caspase-3 activation through theATM/p53 signaling axis. Importantly, TRPM8 inhibition blockade also promoted the upregulation of the NK cell-activating ligand ULBP1, thereby enhancing melanoma susceptibility to NK-mediated cytotoxicity via ULBP1/NKG2D engagement.

CONCLUSIONI

Our findings identify TRPM8 as a novel actionable target in metastatic melanoma. TRPM8 antagonism exerts a dual anti-tumor activity by directly inducing mitochondrial apoptosis and concurrently boosting tumor immune vulnerability to NK cells. These findings suggest that TRPM8 antagonists are promising candidates to overcome treatment resistance and could synergize effectively with current immunotherapies.

A030

THERAPEUTIC POTENTIAL OF SILVER NANOPARTICLES IN AGGRESSIVE BREAST CANCER SUBTYPES: A MECHANISTIC INSIGHT

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INTRODUZIONE

Breast cancer (BC) is the second most commonly diagnosed cancer worldwide. It is classified into three major subtypes: BC expressing hormone receptor (estrogen receptor (ER+) or progesterone receptor (PR+)), BC expressing human epidermal receptor 2 (HER2+) and triple-negative breast cancer (TNBC) (ER−, PR−, HER2−). Traditional therapies face significant challenges, including severe side effects and the development of drug resistance. Silver Nanoparticles (AgNPs) exhibit selective cytotoxicity toward cancer cells, sparing healthy cells, making them attractive candidates for oncological application. This study explores the potential of AgNPs as targeted anticancer agents against TNBC and HER2+ BC.

METODI

AgNPs were synthesized and characterized through spectroscopy UV-vis sprectroscopy, FTIR and NanoSight. Moreover, Trasmission Electron Microscopy (TEM) was employed both to visualize AgNPs morphology and to examine their intracellular effects post-treatment. Two human BC cell lines were utilized: MDA-MB-231 (TNBC) and BT474 (HER2+). Cell viability was assessed using MTT Assay; migration and invasion capabilities were evaluated through Capillary Morphogenesis, Transwell Invasion, and Wound Healing Assays. The molecular mechanisms underlying the effects of AgNPs were investigated through Western Blot, PCR and confocal microscopy.

RISULTATI

AgNPs treatments impaired cell viability and proliferation in both cell lines in a dose- and time-dependent manner. We demonstrated that AgNPs were able to cause the phosphorylation of P38 MAPK in both cell lines, through mitochondrial dysfunction and ROS production. P38 activation triggered CHOP and initiated the intrinsic apoptotic pathway via cytochrome C release and activation of caspase-9/-3. The involvement of P38 was confirmed by SB203580, a selective inhibitor. Conversely, L-BSO, an irreversible glutathione depletor, enhanced ROS levels and further activated the P38 MAPK pathway.

CONCLUSIONI

Our findings demonstrate that AgNPs induce apoptosis in BC cells, primarily through the intrinsic mitochondrial pathway and involving P38 MAPK-mediated stress responses. AgNPs cause mitochondrial membrane depolarization, leading to cytochrome C release and subsequent activation of downstream caspases, resulting in programmed cell death. These results highlight the therapeutic potential of AgNPs in BC treatment, suggesting further in vivo research to confirm their efficacy and safety.

A031

MITOCHONDRIAL ULTRASTRUCTURAL AND MORPHOMETRIC PROFILING OF MONOCYTES IN PATIENTS WITH GERIATRIC FRAILTY

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INTRODUZIONE

Geriatric frailty is a complex syndrome marked by increased vulnerability and declined physiological reserves. While it overlaps with multimorbidity, its distinct biological mechanisms remain poorly understood. To address this, the FRAMITO study investigated mitochondrial function, which results indicate a significant metabolic shift in frail individuals' monocytes, with altered energetics and an impaired association between oxidative metabolism and ROS production. This suggests that a loss of mitochondrial efficiency drives frailty, independent of multimorbidity. To better understand these findings, we extended our analysis to mitochondrial morphology describing our preliminary results here.

METODI

In a restricted sub-cohort of the FRAMITO study, monocytes were isolated from PBMCs via immunomagnetic separation across three distinct patient groups: non-frail without multimorbidity (NFWoM), frail without multimorbidity (FWoM), and frail with multimorbidity (FWM). Mitochondrial morphology was analyzed via transmission electron microscopy. Morphometric data were processed through a comparative workflow involving ImageJ (manual gold standard) and ZEISS ArivisPro software, taking advantage of both manual annotation and AI-based models to identify mitochondrial structural changes associated with frailty and/or multimorbidity.

RISULTATI

Ultrastructural analysis revealed that monocytes from FWoM patients exhibit a distinct mitochondrial profile: organelles are significantly smaller—with reduced area, perimeter, and length—but show increased mitochondrial density compared to NFWoM controls, who displayed larger cytoplasmic volumes. Methodologically, manual quantification was highly consistent across platforms (R=0.98), while the AI workflow showed size-dependent biases (R=0.90). Data collection is ongoing, focusing on the FWM cohort to determine how multimorbidity modulates these alterations.

CONCLUSIONI

These preliminary findings identify mitochondrial morphometric shifts as putative hallmarks of frailty. Such alterations may reflect a specific metabolic and structural reprogramming, paving the way to better define the role of mitochondrial morphology in frailty, whether occurring independently or alongside coexisting multimorbidity.

A032

EXPLORING THE ROLE OF THE CHEMOKINE RECEPTOR CXCR2 IN MUSCLE STEM CELL HETEROGENEITY

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INTRODUZIONE

Muscle stem cells (MuSCs) are a heterogeneous cell population crucial for skeletal muscle regeneration. A key source of MuSC heterogeneity is the cell-to-cell variability in cell surface protein levels, a form of non-genetic heterogeneity, that shapes MuSC fate and regenerative potential. Despite its biological relevance, the molecular basis of non-genetic heterogeneity remains poorly understood. By integrating transcriptional profiling with FACS analysis across distinct MuSC subsets, we identified differential expression of the surface protein CRIPTO, a key regulator of MuSC plasticity. Transcriptomic analyses revealed a marked enrichment of immune-response-related genes in CRIPTO-positive cells, including the chemokine receptor CXCR2 and its ligands.

METODI

Using immunofluorescence (IF), FACS and time-lapse imaging, we characterized CXCR2 surface heterogeneity and interconversion dynamics in primary MuSC subsets. CXCR2 subcellular localization was assessed by IF co-analysis with phospho-ERM (Ezrin/Radixin/Moesin) proteins and vimentin intermediate filaments. Functional assays were further performed following siRNA-mediated knockdown and stimulation with the CXCR2 ligand CXCL2.

RISULTATI

CXCR2 displayed heterogeneous surface expression in primary MuSCs. CXCR2+ and CXCR2- subsets rapidly interconverted, suggesting that CXCR2 marks dynamic and reversible states driven by receptor level fluctuations. At the subcellular level, CXCR2 was organized in discrete patches that colocalized with ERM proteins. CXCR2 patches also colocalized with vimentin intermediate filaments along the shafts of emerging protrusions. These observations suggest that CXCR2 is associated with the cytoskeletal network of proliferating MuSCs. Functionally, siRNA-mediated knockdown of CXCR2 significantly reduced cell velocity, leading to decreased overall displacement compared to controls. Consistently, stimulation with CXCL2 triggered a rapid and transient increase in F-actin intensity.

CONCLUSIONI

These findings identify CXCR2 as a novel marker of MuSC functional heterogeneity and suggest that its dynamic expression contributes to the regulation of cell motility during muscle repair.

A033

THE DNA MINOR GROOVE BINDERS TRABECTEDIN AND LURBINECTEDIN ARE POTENT ANTITUMOR AGENTS IN HUMAN INTRAHEPATIC CHOLANGIOCARCINOMA

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INTRODUZIONE

Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver tumor. Due to its aggressive nature and resistance to conventional treatments, there is a pressing need to develop novel and more effective therapies for this deadly malignancy.

METODI

Here, we explored the therapeutic potential of the DNA minor groove binders trabectedin (TRB) and lurbinectedin (LUR) for the treatment of iCCA using cell lines, spheroids, cancer-associated fibroblasts (CAFs), patient-derived tumor organoids (PDOs), and the chicken chorioallantoic membrane (CAM) in vivo model.

RISULTATI

TRB and, more substantially, LUR, significantly inhibited cell growth in iCCA cell lines, spheroids, CAFs, and PDOs at very low nanomolar concentrations. Specifically, the two drugs significantly reduced proliferation, triggered apoptosis, and caused DNA damage in iCCA cells. At the metabolic level, TRB and LUR decreased mitochondrial respiration and glycolysis. At the molecular level, the two compounds effectively downregulated the mammalian target of rapamycin complex 1 (mTORC1) and Hippo/YAP pathways and suppressed the expression of yesassociated protein 1 (YAP1), cellular myelocytomatosis oncogene (c-Myc), E2F transcription factor 1 (E2F1), Bromodomain-containing protein 4 (BRD4), TEA domain transcription factor 4 (TEAD4), and cluster of differentiation 7 (CD7) proto-oncogenes. Furthermore, LUR significantly restrained the in vivo growth of iCCA cells in the CAM model.

CONCLUSIONI

Our data indicate that TRB and LUR possess strong anti-proliferative and pro-apoptotic activities and could represent promising therapeutic agents for the treatment of iCCA.

A034

ALZHEIMER'S DISEASE ASTROCYTES EXHIBIT ENHANCED SUSCEPTIBILITY TO GEMCITABINE-INDUCED SENESCENCE

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INTRODUZIONE

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by chronic neuroinflammation and progressive cognitive decline. Astrocytes are essential for central nervous system homeostasis, but under pathological conditions, they may acquire a senescent pro-inflammatory phenotype that contributes to disease progression. Cellular senescence, a hallmark of aging, involves cell-cycle arrest, DNA damage, oxidative stress and secretion of pro-inflammatory mediators collectively known as the senescence-associated secretory phenotype (SASP), features linked to AD pathology. Given the emerging role of astrocyte senescence in AD, gemcitabine (GEM), a DNA-damaging chemotherapeutic known to induce therapy-induced senescence (TIS), was used to model senescence-related alterations in astrocytes. We hypothesized that astrocytes derived from AD patients are more susceptible to GEM-induced senescence than those from healthy subjects (HS).

METODI

Human primary astrocytes from HS and AD patients were treated with GEM 0.5 μM for 48 and 72h, followed by 72h in fresh medium (72h+72h). Cell viability was assessed by MTT assay, and apoptosis by Annexin V/PI flow cytometry. Cell-cycle arrest, ROS, and mitochondrial ROS (mtROS) were evaluated by flow cytometry. Cell proliferation was assessed by Ki-67 immunofluorescence. Senescence was evaluated by senescence-associated β-galactosidase (SA-ßGal) activity, macroH2A.1 and γH2AX expression.

RISULTATI

GEM induced moderate cell death in the 72h+72h condition (~30–34%) with no differences between HS and AD astrocytes. Both groups showed proliferative arrest, confirmed by reduced Ki-67 expression and stable cell counts. ROS and mtROS levels increased significantly after 48 and 72 h of treatment in both groups; while at 72h+72h, mtROS levels rose significantly only in AD cells. SA-ßGal activity, macroH2A.1 and γH2AX expression were markedly increased after GEM exposure, with a stronger effect in AD astrocytes (~35%), suggesting greater susceptibility to TIS.

CONCLUSIONI

These findings demonstrate that GEM effectively induces a senescent phenotype in human primary astrocytes and reveal an increased vulnerability of AD-derived astrocytes to TIS. This model may help investigate astrocyte senescence in neuroinflammation and AD progression.

A035

LIFTING THE MIST ON PANCREATIC ISLET CELL DYSFUNCTION IN TYPE 2 DIABETES (T2D): LESSONS LEARNT FROM 1,615 ORGAN DONOR PANCREASES HANDLED AT A SINGLE CENTER.

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INTRODUZIONE

Despite constant efforts to understand the processes that lead to pancreatic islet cell dysfunction in human T2D, several questions remain unanswered, limiting the progression of preventive and therapeutic strategies. We present findings from an extensive, long-term program of islet isolation and characterization aimed at identifying the mechanisms of impaired insulin secretion in T2D.

METODI

From 1995 to 2021, 1,993 organ donor pancreases were received and processed, with data generated with 80% of them. Standardized procedures were used, from pancreas retrieval to islet isolation and from functional studies to molecular analyses.

RISULTATI

Donor (n=1,615) characteristics (median [IQR]) were: age 71 [57-79] years; M/F 54/46%; BMI 25.2 [23.3-27.7] kg/m2; 215 donors had T2D. Compared with non-diabetic (ND) (n=1,390), T2D donors were older and had higher BMI, abdominal circumference and glycemia ($p<0.001$). T2D islet preparations showed reduced insulin secretion (IS) to glucose (G) (~42%), glibenclamide (Glib) (~41%) and, to a lesser extent, arginine (Arg) (~19%). By multivariable regression, T2D was independently associated with lower IS, that was also influenced by age, islet purity, culture time and BMI. In ND islets, G- and Glib- ($p<0.01$) but not Arg-stimulated IS was significantly better with islets from F (n=343) than M (n=374) donors, an effect maintained up to at least 70 years of age. Sex differences were not observed for T2D islets. In diabetic islets, G- and Glib- but not Arg-IS tended to decrease with diabetes duration. Transcriptomic comparison between ND (n=221) and T2D (n=46) islets identified 459 differentially expressed (p adj<0.05, $|FC|>1.5$) genes (181 up- and 278 down-regulated), related to inflammatory and secretory processes by enrichment analyses.

CONCLUSIONI

Large datasets of well-characterized ND and T2D islets are therefore allowing detailed studies of IS and its changes, mainly due to T2D and additional clinical and technical variables. Transcriptomics revealed new genes and pathways in T2D islets. Integration of these data with other omics will help to identify new strategies that specifically target islet cells.

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A036

IDENTIFICATION OF MITOCHONDRIAL CYTOCHROME B (MT-CYB) AS A NOVEL MOLECULAR TARGET OF LAMOTRIGINE IN BREAST CANCER CELLS

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INTRODUZIONE

Breast cancer is a heterogeneous disease comprising several cellular subtypes, including estrogen receptor-positive (ER+) and triple-negative (TNBC) tumors. These subtypes are characterized by high aggressiveness and significant alterations in energy metabolism and cellular bioenergetics. Lamotrigine (LTG), an anticonvulsant drug, has recently shown promising antitumor activity in breast cancer. However, its specific molecular targets and bioenergetic mechanisms remain unclear. This study investigates the mitochondrial cytochrome b subunit (MT-CYB) as a potential primary target of LTG in different breast cancer models.

METODI

Molecular modelling and structural docking were used to analyze the binding affinity of LTG to the active site of MT-CYB. Global proteomic changes were evaluated in lamotrigine-treated MCF-7 cells. For in vitro validation, MCF-7, tamoxifen-resistant MCF-7 (TamR), and MDA-MB-231 cells were treated with LTG at 30 and 50 μM. MT-CYB protein expression was assessed by Western blot, while mitochondrial homeostasis was evaluated by measuring reactive oxygen species (ROS) production, mitochondrial membrane potential, and mitochondrial mass.

RISULTATI

In silico simulations showed that LTG binds to the catalytic site of MT-CYB, interacting with key residues and partially overlapping the reference ligand. Proteomic analysis confirmed LTG's involvement in modulating the mitochondrial respiratory chain, identifying MT-CYB as a direct target. In vitro experiments demonstrated that LTG significantly reduces MT-CYB protein levels in all cell lines tested. Furthermore, LTG induced mitochondrial dysfunction, evidenced by increased ROS production and reduced membrane potential and mitochondrial mass.

CONCLUSIONI

Our results identify MT-CYB as a novel direct molecular target of LTG in breast cancer. The inhibition of this factor, supported by proteomic analysis, in the presence of the drug causes a marked homeostatic failure of the mitochondria, demonstrating that the targeted perturbation of the mitochondrial respiratory chain represents a probable bioenergetic mechanism for its antitumor activity in the various cellular models studied.
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A037

BEYOND HALLMARK STACKING: A DATA-DRIVEN BOTTLENECK FRAMEWORK FOR PRIORITIZING MOUSE LIFESPAN INTERVENTION COMBINATIONS

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INTRODUZIONE

The hallmarks of aging framework organizes aging biology, but does not indicate which interventions should be combined or when they should be applied. We developed a data-driven framework to prioritize bottleneck-aware geroscience combinations using mouse lifespan intervention data.

METODI

Mouse lifespan records were assembled from ITP, DrugAge and RAID/LEV Foundation datasets. Intervention groups were annotated by lifespan outcome, timing, model context, hallmark process and intervention functional domain. Central lifespan effects were based on median/mean lifespan, whereas late-life effects were based on maximum/90th percentile lifespan. Functional domains were linked to pathway and STRING protein-network relationships to classify domain pairs as convergent, network-proximal complementary, distant/orthogonal complementary or weak/uncertain.

RISULTATI

The final database included 207 intervention groups and 586 source-level mouse records. Central and late-life lifespan effects were related but separable, with several interventions improving median/mean lifespan without equivalent late-life tail-shifting. Timing strongly influenced late-life efficacy: very early/developmental and selected late-life/geriatric interventions showed prominent tail-shifting, whereas the adult 6–12 month window contained many neutral or negative interventions. Functional domains differed in effect heterogeneity and timing profile. Network/pathway analysis separated likely redundant domain pairs from complementary relationships. Conservative candidate combinations were enriched for late-compatible, non-germline/non-developmental interventions involving growth-maintenance reallocation, metabolic input modulation, bioenergetic/cofactor support and host-microbiome/barrier modulation.

CONCLUSIONI

Mouse lifespan interventions cannot be interpreted by hallmark identity alone. Late-life lifespan extension depends on lifespan metric, timing, model context and intervention functional domain. Integrating these variables with pathway and STRING network relationships provides a reproducible framework to prioritize rational geroscience combinations.

A038

HIPK2 INHIBITION PROMOTES CYTOPLASMIC ACCUMULATION OF TDP-43 AND ITS INTERACTION WITH VAPB: A PROTEOMIC INSIGHT INTO ALS PATHOGENESIS

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INTRODUZIONE

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by selective loss of motor neurons and impairment of muscle control. A key ALS marker is the cytoplasmic accumulation of TAR DNA-binding protein 43 (TDP-43), an RNA/DNA-binding protein that shuttles between nucleus and cytoplasm and regulates RNA splicing and metabolism.
In our laboratory, we have demonstrated that Homeodomain Interacting Protein Kinase 2 (HIPK2) is a key regulator of TDP-43 homeostasis. In fact, HIPK2 ablation causes cytoplasmic TDP-43 accumulation in human neuroblastoma SH-SY5Y cells, and in neurons of HIPK2 knock-out mice, which display an ALS-like phenotype. To investigate the effects of the TDP-43 mis-localization caused by HIPK2-depletion, we aimed to analyze the TDP-43 interactome and its functional impact on cell physiology.

METODI

We used a proteomic approach to analyze the TDP-43 interactome in HIPK2-interfered (HIPK2i) and control (Ctrli) SH-SY5Y cells. To further investigate the specific interaction identified between TDP-43 and VAPB (VAMP Associated Protein B And C), we performed in silico molecular docking and in vitro fluorescence spectroscopy analyses using recombinant proteins. Furthermore, to evaluate the functional impact of this interaction on cell physiology, we performed Proximity Ligation Assay (PLA) experiments.

RISULTATI

Interestingly, we observed a marked enrichment of mitochondrial- and endoplasmic reticulum (ER)-associated proteins among TDP-43 interactors in HIPK2i cells. In particular, we identified an interaction between TDP-43 and VAPB, an ER membrane protein that plays a crucial role in the formation of mitochondria-ER contact sites (MERCS). In silico and in vitro analyses supported the existence of a direct binding between TDP-43 and VAPB. Notably, PLA data successfully demonstrated a significant reduction in the number of ER-mitochondria contact sites in HIPK2i SH-SY5Y cells, providing solid evidence that the HIPK2/TDP-43 axis directly modulates MERCS stability.

CONCLUSIONI

Taken together, our results reveal a new functional interaction among HIPK2, TDP-43 and VAPB that could be relevant for the pathogenesis of ALS.

A039

EPIGENETIC REGULATION OF LAV-BPIFB4 DRIVES METABOLIC REWIRING AND MODULATES HEALTHY AGING

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INTRODUZIONE

LAV-BPIFB4 is a longevity-associated variant gene involved in the regulation of vascular and immune homeostasis. Initially identified in long-living individuals, it has been linked to protection against cardiovascular and inflammatory diseases. As metabolic and epigenetic reprogramming is increasingly recognized as a key determinant of healthy aging, this study investigated the mechanisms underlying LAV-BPIFB4 activity focusing on its epigenetic regulation and its ability to modulate endothelial metabolism. It has been investigated how these processes influence transcriptional programs associated with inflammation, thrombosis and cellular adaptation providing insights into the molecular basis of resilience to age-related diseases.

METODI

Patient serum samples were analyzed for epigenetically active metabolites to identify potential factors associated with metabolic and inflammatory responses. Transcriptomic analyses were conducted and, in parallel, endothelial metabolic activity was assessed in cell models using Seahorse. The data were organized based on specific stratification criteria. Some histonic modifications associated with metabolic activity were analyzed by Western blot.

RISULTATI

LAV-BPIFB4 induced a differential response between sensitive and resistant samples. In sensitive samples, a reduction in basal respiration was observed while resistant samples showed an increase in basal respiration accompanied by modulation of the proton leak. Transcriptomic analysis identified genes (DGE) and pathways involved in thrombosis, DNA repair, inflammation. In resistant phenotypes, a modulation in acetylation/lactylation markers is also observed, while ketoglutarate metabolism seems to show a genotype dependent effect.

CONCLUSIONI

This work suggests that LAV-BPIFB4 plays a key role in the interplay between metabolic regulation, epigenetic status and transcriptional programs. These findings highlight a potential involvement of LAV-BPIFB4 in disease-related mechanisms, although further studies are required to better define its role in aging and age-associated disorders.

A040

IVERMECTIN AND METHYLENE BLUE ENHANCE CHEMOSENSITIVITY IN RESISTANT CANCER CELL MODELS

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INTRODUZIONE

Cancer remains a major global health challenge due to its complex and multifactorial nature. Despite advances in surgery, radiotherapy, and immunotherapy, patient outcomes have improved, but chemotherapy continues to play a central role in cancer treatment. However, its efficacy is often compromised by drug resistance and severe adverse effects, highlighting the urgent need for novel therapeutic strategies. Drug repurposing represents a promising approach because clinically approved drugs have established safety profiles, lower development costs, and accelerated translational potential. In this context, we investigated the antitumor effects of ivermectin (IVM) and methylene blue (MB), alone and in combination, in resistant cancer models.

METODI

Multiple in vitro models of chemoresistance were used, including the sorafenib-resistant HLE-LPAR6 hepatocellular carcinoma (HCC) cell line and two experimentally generated resistant cell populations: HCT-116-R (colorectal cancer) and Huh-7-R (HCC). Resistance was induced by weekly pulsed exposure to the IC_{50} concentrations of 5-fluorouracil (5-FU) or sorafenib, respectively, over six weeks. Drug sensitivity was assessed using crystal violet assays following 48 hours of treatment. Resistant cells were subsequently treated with IVM and/or MB, either alone or in combination with 5-FU or sorafenib. Cytotoxicity was evaluated by automated cell counting and flow cytometry.

RISULTATI

Both IVM and MB significantly reduced cell viability when administered individually, while combination treatments exerted enhanced cytotoxic effects. Notably, the addition of IVM and MB to standard chemotherapy agents (5-FU or sorafenib) markedly decreased survival of resistant cells compared with single-agent treatments, suggesting a synergistic or additive antitumor effect.

CONCLUSIONI

Our findings indicate that IVM and MB may have therapeutic potential against chemotherapy-resistant cancer cells. These results support further investigation of repurposed drug combinations as innovative strategies to overcome drug resistance and improve anticancer treatment efficacy.

A041

OVERCOMING COLORECTAL CANCER CHEMORESISTANCE THROUGH METABOLIC AND KINASE INTERVENTIONS

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INTRODUZIONE

Resistance to 5-fluorouracil (5-FU) remains a significant challenge in the treatment of colorectal cancer and is often associated with metabolic reprogramming and mitochondrial dysfunction. This study aimed to characterize the metabolic adaptations linked to acquired 5-FU resistance in HCT-116 colorectal cancer cells and to assess the impact of the multikinase inhibitor SGK-1 on 5-FU sensitivity.

METODI

A resistant HCT-116-R cell line was generated via weekly pulsed exposure to 5-FU IC_{50} concentration over six weeks. Drug sensitivity was analyzed using a crystal violet assay post 48-hour treatment. Mitochondrial respiration metrics included basal, ATP-linked, maximal, and non-mitochondrial respiration, measured by a Hansatech oxygraph system. Quantification of intracellular glucose, lactate, ATP, NADH, and total NAD^+/NADH levels was performed. HCT-116-R cells received treatment with SGK-1, alone or with 5-FU (20 or 100 μM), and growth inhibition was evaluated after 48 and 96 hours.

RISULTATI

HCT-116-R cells displayed markedly reduced sensitivity to 5-FU compared with parental cells, with IC_{50} values increasing from approximately 20 μM in parental cells to nearly 400 μM in resistant cells. Resistant cells showed decreased basal respiration and ATP-linked respiration, whereas maximal respiration was largely preserved. In contrast, non-mitochondrial respiration was significantly increased. Metabolic analysis also revealed increased intracellular glucose and lactate accumulation together with reduced NADH and NAD^+/NADH levels, indicating a shift toward a glycolytic phenotype associated with altered redox homeostasis. Treatment with SGK-1 increased sensitivity to 5-FU in HCT-116-R cells, suggesting that kinase-mediated metabolic modulation may contribute to overcoming chemoresistance in colorectal cancer.

CONCLUSIONI

Acquired resistance to 5-FU in HCT-116-R cells is associated with metabolic reprogramming characterized by impaired mitochondrial efficiency, increased non-mitochondrial respiration, and enhanced glycolytic dependence. The effect of SGK-1 in enhancing sensitivity to 5-FU suggests that kinase-dependent metabolic pathways may contribute to colorectal cancer chemoresistance.

A042

INTEGRATIVE SPATIAL ANALYSIS REVEALS CD147-ASSOCIATED TUMOR-MICROENVIRONMENT NICHES IN POSTERIOR FOSSA EPENDYMOMAS

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INTRODUZIONE

Posterior fossa ependymomas (PF-EPNs) comprise two principal molecular subgroups, PFA and PFB, with distinct prognosis. PFA tumors, characterized by EZHIP overexpression and H3K27me3 loss, are predominantly pediatric and aggressive, whereas PFB tumors retain H3K27me3 and show a more favorable clinical course. The spatial organization of tumor–microenvironment interactions in these tumors remains poorly understood. CD147 (BSG), a regulator of metabolic adaptation, extracellular matrix remodeling, and immune interactions, has emerged as a candidate mediator of tumor–microenvironment crosstalk in pediatric brain tumors.

METODI

We integrated single-cell spatial transcriptomics (Xenium; 5 PFA, 5 PFB) with multiplex spatial proteomics (PhenoCycler-Fusion, 40-marker panel) and multiplex immunofluorescence (PhenoImager) in an expanded cohort (12 PFA, 6 PFB). Cell populations were annotated using CIA, while spatial analyses, including proximity measurements and spatial autocorrelation, were performed with HALO.

RISULTATI

Spatial transcriptomics revealed that PFA tumors display a compact niche architecture centered on mesenchymal tumor states (MEC) located near undifferentiated tumor cells (UEC), hypoxic regions, and immune populations, a configuration largely absent in PFB tumors. CD147 expression was enriched in proliferative tumor populations and microglial compartments. CD147/Ki67 co-expression was significantly increased in UEC-A cells in PFA versus PFB and validated at the protein level. Spatial analyses showed that proliferative CD147#PCNA#SOX2# tumor niches preferentially localized near mesenchymal CD147#VEGFA# populations and HIF1A# hypoxic regions, forming a spatially ordered hierarchy that appeared more dispersed in PFB tumors. Transcriptional analyses revealed that CD147-positive tumor cells in PFA were enriched for cell-cycle and stress-adaptive programs, whereas CD147-positive populations in PFB displayed immune-associated signatures. CD147 also delineated distinct microglial states, with CD147-positive microglia in PFA showing endothelial-associated activation programs and closer spatial proximity to CD31# endothelial cells. Finally, CD147 expression spatially coincided with EZHIP-positive tumor compartments and regions of reduced H3K27me3.

CONCLUSIONI

Integrated spatial transcriptomic and proteomic analyses identify CD147 as a marker of coordinated tumor and microenvironmental niche organization in PF-EPNs and highlight spatial programs distinguishing aggressive PFA from PFB tumors.

A043

ALTERNATIVE PET TRACERS FOR THE EARLY DETECTION OF OSIMERTINIB RESISTANCE IN EGFR-MUTATED NSCLC

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INTRODUZIONE

Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases, and EGFR mutations are reported in about 15% of patients. Although the EGFR tyrosine kinase inhibitor osimertinib is associated with high response rates, acquired resistance ultimately limits its long-term efficacy. Resistance mechanisms are heterogeneous and may be either EGFR-dependent or EGFR-independent. ^{18}F-FDG PET/CT is commonly used to predict early treatment response; however, alternative PET tracers may be more suitable for detecting disease progression and emerging resistance.

METODI

Experiments were performed in osimertinib-sensitive (OS) and -resistant (OR) EGFR-mutated NSCLC cell lines. PI3K/AKT pathway was evaluated by Western blotting, glucose consumption was measured by an enzymatic assay, while transport assays were performed using radiolabelled tracers..

RISULTATI

In a small cohort of patients who progressed on osimertinib through EGFR-independent resistance mechanisms, total lesion glycolysis (TLG), assessed by ^{18}F-FDG PET, was reduced compared with baseline, suggesting that ^{18}F-FDG PET may not always accurately predict disease progression during therapy. Preclinical data showed that osimertinib reduced AKT phosphorylation, deoxy-D-glucose,2-[1,2-^{3}H(N)] uptake, and glucose consumption in all OS cells. However, similar effects were also observed in OR cells with EGFR-independent resistance. Preliminary results showed that osimertinib reduced the intracellular transport of ^{14}C-acetate and ^{14}C-methyl-choline in both OS and OR cells, whereas [methyl-^{3}H]-thymidine uptake remained unchanged in OR cells demonstrating higher selectivity versus the resistant phenotype. In vivo experiments in NSG mice are ongoing.

CONCLUSIONI

Overall, our findings indicate that ^{18}F-FDG PET-based metabolic criteria may have limited accuracy in predicting disease progression during TKI therapy in patients with NSCLC. Alternative PET tracers other than ^{18}F-FDG should therefore be considered to improve the early identification of resistance to osimertinib.

A044

SYNERGISTIC THERAPEUTIC POTENTIAL OF COMBINED AXL AND FAK INHIBITION IN OSTEOSARCOMA

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INTRODUZIONE

Osteosarcoma (OS) carries a high mortality rate due to metastatic progression, demanding novel targeted therapies. Since the AXL and FAK pathways critically drive OS chemoresistance and metastasis, dual inhibition could overcome the compensatory mechanisms that limit the efficacy of monotherapies. This study investigates the anti-tumor efficacy of combined AXL (TP-0903) and FAK (Y-15) targeting using 2D/3D in vitro models. Through multi-level molecular and phenotypic profiling, we aim to define the therapeutic potential and mechanistic basis of this novel combinatorial approach for OS.

METODI

In silico analyses explored the biological relevance and synergistic potential of AXL and FAK (PTK2) in OS. In vitro, human OS cell lines cultured in 2D and as 3D spheroids were used to evaluate the anti-tumor effects of Y-15 and TP-0903 assessing cell proliferation, death, and migration by Sulforhodamine B, Trypan Blue, and Wound Healing assays. Transcriptomic profiling was conducted to identify modulated genes, followed by Western blotting to validate the identified targets and investigate the expression and phosphorylation of key signaling and epithelial-to-mesenchymal transition proteins.

RISULTATI

In silico analysis showed that while AXL and FAK lack direct transcriptional correlation, they converge on a Rho-GTPase network regulating cytoskeletal remodeling and migration, providing a strong rational basis for dual targeting. In vitro, both TP-0903 and Y-15 monotherapies reduced OS cell survival in a dose- and time-dependent manner. Notably, their combination demonstrated a significant synergistic effect across multiple OS cell lines, markedly enhancing cell death and apoptosis while drastically impairing cell invasion. Transcriptomic and protein expression analyses highlighted a dysregulation of members of the RhoA family, providing further insight into the synergistic mechanisms and altered signaling pathways involved.

CONCLUSIONI

By demonstrating that the dual inhibition of AXL and FAK exerts a synergistic antitumor effect in OS, this study provides a strong biological basis for developing new and more effective therapeutic strategies to improve clinical outcomes in patients with OS.

A045

ALTERED OREXINERGIC TRANSMISSION PROMOTES AGE-RELATED NEUROINFLAMMATION BY INDUCING A LIPID-DROPLET ACCUMULATING PHENOTYPE IN MICROGLIA

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INTRODUZIONE

Microglia is a key regulator of brain immunity, and its dysfunction is heavily implicated in aging and neurodegeneration. Aging-associated microglia exhibit extensive lipid droplet (LD) accumulation, impaired phagocytosis, and a pro-inflammatory phenotype, collectively defining "lipid droplet-accumulating microglia" (LDAM). Although previous studies have linked orexin A (OxA) signalling to metabolic regulation, and evidence shows an upregulation of orexinergic transmission in aging, the precise role of OxA via orexin receptor type1 (OX1R) in the onset of LDAM remains unexplored. Our aim is to investigate whether OxA/OX1R signalling contributes to the development of the LDAM phenotype during aging by promoting LDs accumulation and microglial dysfunction.

METODI

To investigate the effects of aging-related hyperorexinemia on microglia, we employed complementary experimental models. Aged mice (>550 days) were used to evaluate endogenous LD accumulation in acute hippocampal brain slices through immunofluorescence. To dissect the underlying pharmacological mechanisms, BV2 cells and primary mixed glial cultures obtained from fluorescent CX3CR1-eGFP mice were used as in vitro models. Cells were exposed to OxA in the presence or absence of the OX1R antagonist SB-334867 and the DGAT-1 inhibitor PF-06424439 (to block LD synthesis). LD accumulation was quantified, phagocytosis was monitored via Incucyte Zoom live-cell imaging, and cytokine release was assessed through multiplex ELISA.

RISULTATI

Pathological elevations of OxA induced a dysfunctional microglial state reminiscent of LDAM. In both BV2 and CX3CR1-eGFP primary microglia, OxA significantly increased LD accumulation and impaired phagocytosis in a dose- and time-dependent manner, while triggering a pro-inflammatory cytokine profile. Mechanistically, these alterations were mediated by OX1R signaling; pharmacological blockade of OX1R via SB-334867 or inhibition of triglyceride synthesis via DGAT-1 fully restored microglial lipid homeostasis and phagocytic function. In vivo, chronic OxA exposure in young mice successfully recapitulated the LDAM-like morphology and functional defects natively observed in aged microglia.

CONCLUSIONI

These findings identify hyperorexinemia as a novel metabolic driver of microglial dysfunction via OX1R-mediated lipid dysregulation. This work establishes a direct link between altered orexinergic transmission and age-associated neuroinflammation, highlighting OX1R as a potential therapeutic target to rescue brain immune surveillance.

A046

TARGETING LAT1 UNCOVERS A METABOLIC–EPIGENETIC VULNERABILITY AND ENHANCES BNCT POTENTIAL IN GLIOBLASTOMA

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INTRODUZIONE

Glioblastoma (GBM) is the most lethal primary brain tumor, driven by metabolic adaptability, therapeutic resistance, and continuous progression. Emerging evidence highlights a close link between metabolic rewiring and epigenetic remodeling in sustaining GBM aggressiveness. The L-type amino acid transporter 1 (LAT1), highly expressed in GBM, is a key regulator of nutrient uptake and mediates the transport of boron-containing compounds used in Boron Neutron Capture Therapy (BNCT). This study aimed to investigate the contribution of LAT1 to metabolic and epigenetic regulation in GBM and to assess its potential impact on boron accumulation and BNCT responsiveness.

METODI

LAT1 function was assessed in GBM cellular models through genetic silencing and pharmacological modulation using histone deacetylase inhibitors (HDACis). Tumorigenic properties were evaluated by proliferation, clonogenic, and spheroid formation assays. Integrated transcriptomic, metabolic, and protein analyses investigated LAT1-dependent pathways and metabolism–epigenetic crosstalk. Boron uptake was quantified by mass spectrometry in shLAT1 models.

RISULTATI

LAT1 depletion suppressed GBM growth, reducing proliferation, clonogenic capacity and spheroid expansion. Transcriptomic profiling showed downregulation of glycolytic, hypoxia-related and adaptive stress-response pathways linked to tumor progression. Consistently, metabolic analyses demonstrated impaired glycolytic activity following LAT1 silencing. LAT1 loss reduced the expression of key metabolic effectors, including HKII and ACLY, alongside decreased global histone acetylation, linking amino acid transport, metabolic regulation and chromatin remodeling. Moreover, LAT1 silencing reduced boron accumulation, while HDACi increased its expression, suggesting an epigenetic strategy to enhance BNCT responsiveness through epigenetic intervention.

CONCLUSIONI

This study identifies LAT1 as a key regulator of the metabolic–epigenetic network sustaining GBM aggressiveness. Beyond tumor metabolism, LAT1 influences boron uptake and may modulate BNCT efficacy. These findings support LAT1 as a promising therapeutic target and predictive biomarker for precision medicine approaches combining metabolic, epigenetic and boron-based therapies in GBM.

A047

“TARGETING HYPOXIA PATHWAYS IN BREAST CANCER CELL LINES: THE EFFECTS OF NOVEL HIF LIGANDS”

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INTRODUZIONE

Triple-negative is the most aggressive and highly hypoxic subtype of breast cancer. Hypoxia is a critical driver of tumour progression and in this condition, hypoxia-inducible factors (HIFs), particularly HIF-1α and EPAS1, become stabilized and transcriptionally active. These factors enhance angiogenesis, metabolic reprogramming, epithelial–mesenchymal transition (EMT), cell survival, thus promoting tumor adaptation and progression.
One of the most relevant oncogenic pathways involves the regulation of HIF-1α. The present study aims to evaluate the effects of newly synthesized chemical compounds targeting HIF signaling on the expression of hypoxia-related markers and key tumorigenic processes. These effects were investigated across different breast cancer cell lines in order to assess the potential of these compounds as novel therapeutic agents.

METODI

The compounds were evaluated in vitro using the MDA-MB-231 and MCF-7 breast cancer cell lines to assess their effects on cell proliferation and viability. A Western blot analysis was performed to evaluate the expression levels of key markers involved in the hypoxic response, EMT, autophagy, and apoptosis. The subcellular localization of HIF-1α and EPAS1 was investigated by immunofluorescence assays.

RISULTATI

Treatment with the selected compounds significantly reduced cell viability and proliferation, as well as the expression of HIF-1α and HIF-2α. A modulation of EMT markers was also observed. Analysis of the autophagic and apoptotic pathways revealed a decrease in the expression of the relevant markers, alongside an increase in apoptotic markers. Immunofluorescence analysis further supported the Western blot findings, showing a reduction in HIF-1α and EPAS1fluorescence intensity.

CONCLUSIONI

Our findings support the therapeutic potential of HIF-targeting compounds across different breast cancer cell lines. By disrupting hypoxia-mediated signaling pathways, these agents may reduce tumor proliferation and invasiveness, thereby enhancing treatment response. Further studies using 3D models will be conducted to validate these results in systems that more closely recapitulate the in vivo pathological context.

A048

SINGLE-CELL RNA SEQUENCING REVEALS TRANSCRIPTIONAL HETEROGENEITY IN RAT HEPATIC CELL LINES: TOWARDS THE IDENTIFICATION OF DRUG RESISTANCE-ASSOCIATED SUBPOPULATIONS IN HCC

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INTRODUZIONE

Hepatocellular carcinoma (HCC) represents the third leading cause of cancer-related deaths worldwide. Despite recent advances in systemic therapies, including multikinase and immune checkpoint inhibitors, clinical responses remain limited to a subset of patients and therapeutic resistance remains a major challenge. Cellular heterogeneity is recognized as a key driver of resistance to conventional therapies. Therefore, identifying specific cell subpopulations and characterizing their transcriptional heterogeneity are crucial for understanding tumor progression, identifying new prognostic markers and therapeutic targets.

METODI

We performed single-cell RNA sequencing on two immortalized rat hepatocyte cell lines, RH and RNT, previously established and characterized in our laboratory from rat liver samples subjected to the Resistant Hepatocyte rat model. RNT cells are non-tumorigenic and resemble normal liver tissue, whereas RH cells are fully transformed and display an HCC-like transcriptional profile. Single-cell capture and barcoding were performed using the 10x Genomics Chromium IX platform, followed by library preparation and Illumina high-throughput sequencing. Raw data were processed with Cell Ranger for alignment, while clustering was performed using Loupe Browser.

RISULTATI

As expected, clustering analysis revealed a marked transcriptional heterogeneity, with two distinct populations, indicative of a strong separation between RH and RNT cells. In addition to the transcriptional differences observed between the two cell lines, both exhibited intra-cell line heterogeneity. Notably, RNT cells displayed a higher degree of heterogeneity, with 11 distinct clusters, compared with 7 identified in RH cells. Gene function analysis revealed significant differences in biological processes related to cell proliferation, migration, invasion and metabolic reprogramming.

CONCLUSIONI

These findings demonstrate that both cell lines exhibit transcriptional heterogeneity at the single-cell level. Distinct subpopulations may contribute differentially to drug treatment, representing a potential mechanism underlying therapeutic resistance in HCC. Ongoing pathway enrichment and GSEA analyses aim to identify cluster-specific transcriptional signatures. Overall, this study highlights single-cell transcriptomics as a powerful strategy to investigate heterogeneity in hepatic cell line models, with the ultimate goal of identifying specific subpopulations associated with drug resistance in HCC.

A049

“SIRT3 AND SIRT5 MODULATION IN METABOLIC REPROGRAMMING OF HCC”

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INTRODUZIONE

The metabolic reprogramming of cancer cells is a distinguished feature that is pivotal to their survival and proliferation. The most well-established metabolic changes are the shift towards aerobic glycolysis and glutamine consumption in order to meet the high energy and carbon substrates requirements needed for growth. Less is known about the lipid metabolic reprogramming in carcinogenesis. Sirtuins are a family of seven NAD dependent histone deacetylase enzymes regarded as metabolic sensors. Mitochondrial SIRT3 and SIRT5 play a crucial role in the adaptation of the cellular metabolism, promoting Fatty Acid Oxidation (FAO) and Urea Cycle (UC), both of which are generally de-regulated in cancers, such as Hepatocellular Carcinoma (HCC). The modulation of mitochondrial sirtuins activity may represent a novel therapeutic route to be investigated. The project aims to evaluate the role of mitochondrial sirtuins SIRT3 and SIRT5 in the lipid metabolic reprogramming that occurs in hepatic carcinogenesis.

METODI

Human hepatoma cells HepG2 were treated with MC2791 and MC3138, novel selective activators of SIRT3 and SIRT5 respectively, both alone and in combination. Cell viability and clonogenicity were measured by Trypan blue exclusion assay and crystal violet staining. Protein expression was analysed by western blot and immunofluorescence. Organelles were stained by lysotracker and mitotracker and mitochondrial activity was assessed by JC-1 staining. Lipid droplets presence by Red Oil O staining.

RISULTATI

We observed the reduction of cancer cell viability and clonogenicity following the treatment and the downregulation of the autophagy and mitophagy processes through the reduced expression of corresponding markers and enhanced mitochondrial membrane potential. Urea cycle and lipid metabolism were also re-established with an increased expression of CPS-1 and a reduction of lipid droplets number and size.

CONCLUSIONI

Our results suggest that SIRT3 and SIRT5 modulation may be a potential therapeutic strategy to revert the metabolic reprogramming of HCC cells.

A050

DISRUPTING AUTOPHAGY-MEDIATED CHEMORESISTANCE IN GLIOBLASTOMA: KMT5A AS A NOVEL THERAPEUTIC VULNERABILITY

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INTRODUZIONE

Glioblastoma (GB) is the most aggressive primary brain tumor in adults and remains largely unresponsive to current therapies. Although MGMT promoter methylation predicts increased sensitivity to temozolomide (TMZ), patient prognosis remains poor due to therapy resistance. We recently demonstrated that the lysine methyltransferase KMT5a is overexpressed in glioblastoma and correlates with worse clinical outcome (Della Monica et al.). KMT5a catalyzes H4K20 monomethylation, a key epigenetic mark involved in DNA damage repair and genome stability.Inhibition of KMT5a in established and primary GB cell lines induces DNA damage accumulation, proliferative arrest, and impairment of autophagic flux. Since TMZ activates autophagy as a cytoprotective mechanism, we hypothesized that KMT5a inhibition could enhance TMZ efficacy by blocking this adaptive survival pathway and preventing the emergence of resistant clones.

METODI

MGMT methylated glioblastoma cell lines and patient-derived primary GB cultures were treated with TMZ and KMT5a inhibitor alone, or in combination therapy. Cell viability and proliferation were assessed through clonogenic assays. DNA damage accumulation was evaluated by γH2AX immunodetection.Cell death was monitored using viability caspase assays. Long-term chronic treatment experiments were performed to evaluate the emergence of TMZ-resistant clones under single versus combined treatment conditions

RISULTATI

KMT5a inhibition induced DNA damage accumulation, reduced proliferative capacity, and impaired autophagic flux in both established and primary GB models. Combined treatment with TMZ significantly enhanced GB cell death compared to monotherapy, particularly in MGMT-methylated cells, and markedly reduced the emergence of TMZ-resistant clones during chronic treatment. Overall, these findings suggest that KMT5a inhibition enhances TMZ efficacy by increasing DNA damage and suppressing autophagy-mediated adaptive resistance mechanisms in glioblastoma cells.

CONCLUSIONI

Our findings identify KMT5a as a promising therapeutic target in glioblastoma and provide a strong preclinical rationale for combining KMT5a inhibitors with temozolomide in MGMT-methylated GB patients. KMT5a inhibition may represent a novel strategy to improve TMZ responsiveness and limit the development of resistance.

A051

A TUMOR-AGNOSTIC NETWORK MEDICINE FRAMEWORK REVEALS TOPOLOGICAL REWIRING OF SYSTEMIC T-CELL IMMUNITY IN LONG-SURVIVING CANCER PATIENTS

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INTRODUZIONE

A particular subset of patients treated with immune-checkpoint inhibitors (ICIs) consistently shows long-term survival, however, the immune mechanisms behind such phenomena remain largely unknown. We propose a network medicine framework that reliably uncovers non-obvious patterns among target T-cell subgroups in flow-cytometric data. Our objective is to investigate the topological rewiring of T-cell response in long survivors (LS) across different tumor cohorts, to uncover a tumor-agnostic immune signature.

METODI

Peripheral blood mononuclear cells were collected from 93 cancer patients undergoing ICI therapy and 52 healthy donors; 31 T-cell subsets were analyzed using flow-cytometry. Patients were considered: LS (OS>18/24), Early Progressors (EP) (PFS≤3) or Intermediate (INT: not EP, not LS). To investigate immune signatures among subgroups, we implemented a differential overlay pipeline: networks were validated for stability and significant rewiring across clinical outcomes (bootstrap resampling; permutation testing). Stable edges were then topologically classified (conserved, specific, or inverted).

RISULTATI

A Comparison of clinical outcomes revealed a profound topological shift. While the EP network is highly centralized around the activatory CD3+PD1+Effector T subset (degree=11), both LS and INT patients lose this activation centrality (degree drops to 5). However, while INT patients lose this signature, only the LS network undergoes extensive structural rewiring to establish a costimulatory, memory-driven topology. In fact, the LS network is characterized by CD3+CD137+Central memory subset ass the central hub (degree=12). This node-centric divergence is driven by statistically robust edge inversions, prominently the interactions between CD3+CD137+Effector memory RA+ and CD3+PD1+Naive (p=0.0015 in EPvsLS), and between CD3+Central memory and CD3+Effector memory (p=0.0065 in EPvLS), proving that long-term survival relies on reversing specific immunological axes rather than global immune restoration.

CONCLUSIONI

From our results, long-term survival seems supported by rewired CD137+ memory T-cell networks. Our framework demonstrates that integrating edge inversions and nodal metrics from flow-cytometry data provides deep biological insights invisible to standard univariate analyses.

A052

HMGA1 LINKS INSULIN RESISTANCE TO NEURODEGENERATION: INSIGHTS FROM OMICS AND TRANSLATIONAL MODELS

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INTRODUZIONE

Insulin resistance (IR) is increasingly implicated in neurodegeneration, yet the mechanisms linking metabolic dysfunction to tau pathology remain unclear. High mobility group A1 (HMGA1), a chromatin architectural factor required for insulin receptor (INSR) transcription, may integrate genetic and metabolic influences through regulation of the tau-encoding gene (MAPT). We investigated whether HMGA1 deficiency promotes IR-associated tau dysregulation and whether this pathway responds to metabolic and pharmacological intervention.

METODI

We combined human post-mortem brain transcriptomics (bulk and single-nucleus RNA-seq), in silico and luciferase analyses of HMGA1 binding to MAPT regulatory regions, differentiated SH-SY5Y neurons with experimental HMGA1 manipulation, clinical/genetic assessment of reduced HMGA1 function, and in vivo studies in Hmga1-KO mice and high-fat diet (HFD)-fed obese mice treated with an insulin-sensitizing agent.

RISULTATI

Human brain RNA-seq analysis revealed an inverse association between HMGA1 and MAPT expression across neuronal and glial populations. Stable MAPT expression across Alzheimer's disease stages suggested early transcriptional regulation. HMGA1 binding motifs were identified within repressive MAPT promoter regions. In differentiated SH-SY5Y neurons, reduced HMGA1 and INSR expression was associated with increased intracellular and extracellular tau/p-tau. Experimental HMGA1 manipulation induced reciprocal tau changes, while promoter assays demonstrated dose-dependent MAPT repression mediated by direct HMGA1-DNA interaction. Clinical and genetic analyses linked reduced HMGA1 function to IR and tau-related neurodegeneration. Consistently, Hmga1-KO mice exhibited hyperglycemia, reduced Insr expression, and increased tau/p-tau load. Importantly, prolonged insulin-sensitizing treatment in HFD-fed obese mice increased cerebral HMGA1 and reduced tau levels, supporting pharmacological reversibility of the HMGA1-tau axis.

CONCLUSIONI

HMGA1 emerges as a metabolically responsive transcriptional repressor of MAPT linking insulin signaling to tau homeostasis and neurodegeneration. Findings from insulin-sensitized obese mice, together with concordant in vitro data, identify HMGA1 as a potential neuroprotective target.

A053

MICROBIOTA REGULATION OF FUNGAL INFECTION AND IMMUNITY VIA TRYPTOPHAN

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INTRODUZIONE

Increasing evidence highlights the crucial role of the microbiota in shaping host immune and metabolic functions, making host–fungus interactions more complex than previously recognized. A dynamic interplay among host, fungi, and microbiota determines the outcome of fungal infections. In particular, microbial dysbiosis predisposes individuals to chronic fungal diseases at both local and systemic levels. Understanding the metabolic and immunological contribution of microbiota may therefore reveal novel therapeutic targets. This study investigated the role of microbiota-derived metabolic pathways in Aspergillus fumigatus infection, particularly in cystic fibrosis patients.

METODI

The dynamics and diversity of bacterial populations were analyzed in both human and murine airways during Aspergillus fumigatus infection. Microbiota composition and metabolite profiles were evaluated using a Transkingdom Network Analysis framework to identify correlations between microbial communities and metabolic pathways. Particular attention was given to the partitioning and metabolism of the aromatic amino acid L-tryptophan and its role in host–fungus–microbiota interactions.

RISULTATI

A substantial increase in bacterial diversity was observed in Aspergillus-infected mice and in patients at risk of infection. Integrative analysis of microbiota metagenomics and metabolomic profiles identified functional bacterial nodes associated with the modulation of host immune responses and fungal pathogenicity. In particular, host–microbiota partitioning of L-tryptophan emerged as a key regulatory mechanism in the tripartite interaction among bacteria, fungi, and the mammalian host, suggesting significant implications for fungal disease progression and immune regulation.

CONCLUSIONI

These findings demonstrate that microbiota-mediated tryptophan metabolism plays a pivotal role in regulating fungal infection and host immunity. The identification of microbiota-dependent metabolic pathways may provide innovative therapeutic targets for the prevention and treatment of Aspergillus fumigatus infections, especially in cystic fibrosis patients.

A054

MODELING ENTERIC HYPEROXALURIA IN A MICROPHYSIOLOGICAL SYSTEM AND TESTING OF MICROBIAL METABOLITES AS POTENTIAL PROTECTIVE AGENTS

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INTRODUZIONE

Hyperoxaluria is characterized by elevated urinary oxalate, leading to calcium oxalate deposition in the kidneys and, in severe cases, systemically. It may be primary—due to inherited defects in hepatic glyoxylate metabolism—or secondary, often arising from conditions with fat malabsorption that increase intestinal oxalate uptake. While advances have clarified primary hyperoxaluria pathogenesis, the heterogeneity of secondary (enteric) hyperoxaluria has hindered therapeutic progress. Modeling enteric hyperoxaluria in a microphysiological system could improve understanding and enable new treatments.

METODI

In this work, we attempted to model secondary hyperoxaluria in a commercial microphysiological system (HUMIMIC Chip4, TissUse GmbH, Berlin, Germany) that contains two different circuits, i.e. a surrogate blood circuit and a urine circuit, and interconnects up to five different organs. In an initial setting, we modeled the kidney by seeding human kidney-derived podocytes cell line PODO/TERT256 and human renal proximal tubular epithelial cell line RPTEC/TERT1 (Evercyte, Vienna, Austria) in the glomerular and tubular compartment, respectively. In the blood circuit, Caco2 cells were cultured in a transwell system to mimic the intestinal barrier.

RISULTATI

Oxalate administration in the intestinal compartment resulted in absorption and excretion in the urine, an effect that was enhanced by treatment with Dextran Sulfate Sodium (DSS), a chemical commonly used to model inflammatory bowel disease. The increased intestinal barrier permeability by DSS and the counteracting effects of microbial metabolites allows for evaluation of a microbial-based therapeutic approach. Further development of the microphysiological system with the addition of the liver compartment would allow to recapitulate the whole-organism oxalate handling in the perspective of developing therapeutic strategies to prevent excessive oxalate intake and/or promoting oxalate secretion.

CONCLUSIONI

In conclusion, a multi-organ-on-a-chip microphysiological system can reproduce oxalate homeostasis along the gut–kidney axis and be used to model intestinal barrier perturbations involved in enteric hyperoxaluria. Microbial metabolites attenuate the inflammatory response in oxalate-exposed renal cells and, together with their protective effects on the intestinal barrier, may disrupt the pathogenic gut–kidney axis at multiple levels.

A055

DISSECTING CIRCADIAN REPROGRAMMING OF THE GUT IN A MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

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INTRODUZIONE

Heart failure with preserved ejection fraction (HFpEF) is a multiorgan clinical syndrome with several tissue dysfunctions, including alterations of intestinal morphology, permeability, and absorption. The circadian clock is an endogenous time-keeping system encompassing virtually every aspect of human physiology. At organismal level, an integrated network of tissue-specific endogenous clocks ensures a tightly coordinated diurnal physiology across the whole body. Circadian misalignment has been linked to a variety of diseases, from metabolic syndrome to cardiovascular disease. Here we hypothesize that in the pathological setting of HFpEF, circadian physiology of the intestine is disrupted, negatively affecting its function, further worsening the patients' quality of life.

METODI

To verify our hypothesis, we have exploited a mouse model of cardiometabolic HFpEF that consists of feeding adult mice with a high fat diet and the NOS inhibitor L-NAME. First, to explore clock function we have performed RNA-seq analysis at 6 time-points along the daily cycle in the intestine. Then, to investigate the physiological relevance of diurnal transcriptional reprogramming in HFpEF, a functional assessment of intestinal tracts has been performed at two circadian times, including microbiological, histological and gene expression analysis, and multiplex cytokine profiling.

RISULTATI

RNA-seq DryR analysis classified oscillatory genes into 14 different models based upon their rhythmicity in each diet condition. Between them, models 4 and 5 are among the most interesting groups, which include genes gaining or losing oscillation in the HFD+L-NAME group, respectively. Functional analysis revealed both time-dependent and disease-dependent changes in bacterial translocation, intestinal inflammation and gene expression.

CONCLUSIONI

Our results so far confirm the main hypothesis that HFpEF is associated with organ-specific circadian reprogramming. Indeed, cardiovascular pathology can itself act as a driver of systemic circadian disruption and altered signaling can drive from the diseased heart to distal tissues, like the intestine. Further ongoing studies aim to clarify the molecular mechanisms driving this circadian reprogramming, as well as to delve deeper into the contribution of the gut microbiota and its metabolites.

A056

THE GLUCOCORTICOID RECEPTOR AS A CONTEXT-DEPENDENT REGULATOR OF METABOLIC REPROGRAMMING AND FATE PLASTICITY IN OVARIAN CANCER

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INTRODUZIONE

Ovarian cancer (OC) cellular heterogeneity is linked to distinct morphological, metabolic, and phenotypic states, with the glucocorticoid receptor (GR) acting as a master regulator of this plasticity. High GR expression correlates with mesenchymal features and distinct metabolic reprogramming, providing a new framework for classifying OC subpopulations and identifying therapeutic targets.

METODI

In vitro analyses were performed using established OC cell lines (including SK-OV-3 and OVCAR-3) and patient-derived cells. GR expression and activity were modulated through genetic and pharmacological approaches followed by isolation and characterization of distinct subpopulations through morphological, phenotypic, and metabolic profiling. These findings were validated using 3D tumor spheroid models to simulate in vivo architecture and cellular interactions.

RISULTATI

Distinct OC cell subpopulations displaying different morphological features were identified and associated with differential GR expression and activity. GR-low populations exhibited a more homogeneous epithelial-like morphology, whereas GR-high populations showed greater heterogeneity, including mesenchymal-like subpopulations. GR-high cells also displayed increased expression of many mesenchymal markers (FN1, COL1A1, N-cadherin) together with transcriptomic signatures related to anti-inflammatory pathways, ECM remodeling, and collagen processes. Further studies showed that glycolysis inhibition induced an epithelial-to-mesenchymal transition (EMT), suggesting that GR may regulate cellular plasticity in ovarian cancer through metabolic reprogramming.

CONCLUSIONI

This study demonstrates that morphological heterogeneity in ovarian cancer reflects distinct biological and metabolic states. Integrating morphological, phenotypic, and metabolic profiling establishes a robust framework for classifying specific tumor cell subpopulations. Ultimately, these insights deepen the understanding of tumor heterogeneity and offer new avenues for developing targeted therapies.

A057

TARGETING ENDOPLASMIC RETICULUM AND GOLGI-DEPENDENT SIGNALING PATHWAY IN MEDULLOBLASTOMA STEM CELLS AS A GATEWAY TO STEM CELL TARGETABILITY

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INTRODUZIONE

Medulloblastoma (MB) is the most common malignant pediatric brain tumor and is frequently associated with resistance to conventional therapies. Among the heterogeneous tumor cell populations, medulloblastoma stem cells (MBSCs) have been identified as key drivers of tumor aggressiveness, therapeutic refractoriness, recurrence, and poor clinical outcome. Consequently, the identification of compounds capable of selectively targeting cancer stem cell (CSC)-associated pathways represents a major unmet clinical need. Brefeldin A (BFA), a Golgi-disrupting agent, has been shown to impair CSC phenotypes by affecting CD133 processing and modulating downstream oncogenic signaling. However, the limited clinical applicability of BFA highlights the need to investigate structurally related molecules with potentially improved therapeutic profiles.

METODI

The biological effects of BFA and its structural analogues, Golgicide A (GCA) and Exo2, were investigated in MBSCs models. Functional and molecular consequences were assessed through stemness-associated markers, clonogenic and sphere forming capability, apoptosis and modulation of oncogenic signaling pathways.

RISULTATI

Among the tested BFA-analogues, GCA most closely reproduced BFA-induced the phenotypic and molecular effects induced by BFA. In particular GCA promoted CD133 cleavage, reduced the expression of stemness markers, impaired sphere-forming and clonogenic potential, and increased the expression of apoptotic factors. These effects were markedly stronger than those observed following Exo2 treatment. Furthermore, both BFA and GCA significantly suppressed the oncogenic PI3K/AKT/mTOR signaling pathway.

CONCLUSIONI

Our findings identify GCA as a structural and functional analogue of BFA capable of effectively targeting CSC-associated pathways in MBSCs. Modulation of CD133 processing and inhibition of oncogenic signaling pathways emerge as promising therapeutic strategies for MB. These results support the further preclinical evaluation of GCA as a potential candidate for CSC-directed therapies in MB.

A058

UNVEILING THE PATHOGENETIC MECHANISMS OF GOLD NANOPARTICLE TOXICITY: THE INTERPLAY OF SIZE, SURFACE CHEMISTRY, AND CELLULAR RESPONSE

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INTRODUZIONE

Gold nanoparticles (AuNPs) are widely investigated for biomedical applications; however, their potential to trigger adverse pathogenetic pathways remains a major safety concern. The molecular mechanisms driving AuNP-induced cellular stress and inflammation are strictly governed by their physical dimensions coupled with the surface chemical/electronic state. Nevertheless, the biophysical surface properties responsible for driving adverse pathogenetic and inflammatory responses remain to be fully elucidated. The CheMatSustain project, funded by the European Union's Horizon Europe research and innovation program (Grant Agreement No. 101137990), investigates how variations in AuNP size and in the type of stabilizer, specifically sodium citrate and polyethylene glycol (PEG), dictate specific pathogenetic outcomes in vitro.

METODI

AuNPs of different sizes were functionalized with either sodium citrate or PEG. To highlight structure–activity relationships, surface chemical and electronic states were characterized by X-ray and Ultraviolet photoelectron spectroscopy (XPS/UPS), coupled with a novel artificial intelligence (AI)-driven data analysis model. Pathogenetic mechanisms were investigated in vitro by assessing cytotoxicity and TLR4-mediated inflammatory markers activation (IRAK1, miR-146a, and IL-6).

RISULTATI

Depending on surface/size/stabilizers, AI-powered XPS/UPS analysis revealed that AuNPs exhibited quantum size effects and different valence band profiles. Depending on AuNPs' pathogenetic response, cytotoxicity and modulation of inflammatory markers suggest their ability to interact differently with cellular metabolism and molecular pathways.

CONCLUSIONI

Our findings demonstrate that the pathogenetic response induced by AuNPs are dependent on the surface characteristics of the nanoparticle. The synergistic convergence between surface physics (AI-powered XPS/UPS) and molecular profiling may bridge the gap between materials science and translational medicine. Integrating XPS/UPS characterization with toxicological profiling provides insights into the molecular mechanisms of nanomaterial toxicity, offering a predictable framework for the design of safer, non-pathogenic nanotherapeutics.

A059

MC2494 SUPPRESSES A CONSTITUTIVE SUB-LYTIC NLRP3 INFLAMMASOME STATE IN ACUTE MYELOID LEUKEMIA

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INTRODUZIONE

The NLRP3 inflammasome promotes caspase-1-dependent GSDMD cleavage and IL-1β maturation. Although classically linked to pyroptotic lysis, our study investigates whether AML cells and patient-derived AML PBMCs display a constitutive, sub-lytic NLRP3-inflammasome activity and whether the pan-sirtuin inhibitor MC2494 can suppress this inflammatory leukemic state.

METODI

NLRP3-inflammasome activation was investigated in AML cell lines and ex vivo AML PBMCs by western blot analysis of upstream and downstream markers, ASC-NLRP3 complex assessment, viability/cell-death assays and inflammatory readouts. MC2494 effects were evaluated across time points and integrated with proteomic profiling, NF-κB pathway analysis, NEK7 evaluation, calcium/homeostasis-related markers and validation in THP-1-derived M1 macrophages.

RISULTATI

AML models showed basal NLRP3-inflammasome competence, with ASC-NLRP3 complex formation and downstream signaling involving caspase-1, GSDMD processing and IL-1β maturation, without massive loss of viability, supporting a sub-lytic inflammatory state. Ex vivo AML PBMCs also displayed basal NLRP3 expression/activation features, indicating that this phenotype is not restricted to established cell lines. MC2494 reduced NLRP3 expression and attenuated downstream inflammasome signaling, limiting GSDMD activation and IL-1β maturation. Time-course proteomics revealed progressive suppression of an NLRP3-related inflammatory module upon MC2494 treatment. Mechanistically, MC2494 impaired NF-κB-dependent priming, reduced NEK7 and affected calcium/homeostasis-associated pathways. Its anti-inflammatory effect was further supported in THP-1-derived M1 macrophages. In patient-derived AML PBMCs, MC2494 consistently suppressed NLRP3 protein levels, apparently independently of mutational background and immunophenotypic state.

CONCLUSIONI

These findings identify a constitutive, sub-lytic NLRP3-inflammasome activity as a relevant inflammatory feature of AML cells and primary AML PBMCs. MC2494 suppresses this program through coordinated inhibition of NLRP3 priming, downstream signaling and associated homeostatic pathways, supporting its potential as a translational tool to target inflammasome-driven inflammation in AML.

A060

EXPLORING THE THERAPEUTIC POTENTIAL OF HISTONE DEACETYLASE 11 (HDAC11) IN DIABETIC CARDIOMYOPATHY AND HEART FAILURE

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INTRODUZIONE

Diabetic cardiomyopathy (DCM) is a major cause of heart failure and mortality in diabetes, independently of other cardiovascular risk factors. Epigenetic mechanisms contribute to DCM by regulating gene expression, metabolism, and stress responses. This study investigated diabetic cardiac injury, focusing on histone deacetylase 11 (HDAC11), its transcript isoforms, and potential regulation by microRNAs and N6-methyladenosine (m6A).

METODI

Computational analyses of HDAC family members and m6A regulators were performed using RNA-seq data from high-glucose–treated AC-16 cardiomyocytes, integrated with GEPIA/GTEx, m6A-Atlas, and UCSC Genome Browser resources to evaluate HDAC11-associated m6A regulation and transcript isoforms. An in vitro model of diabetic cardiomyopathy and heart failure–like injury was established in AC-16 cells exposed to high glucose for 48 h, followed by doxorubicin for 24 h at the experimentally determined IC50. HDAC11 expression/localization and PBMC-derived intracellular/circulating miRNA profiles were assessed in clinically stratified cohorts.

RISULTATI

High-glucose exposure significantly upregulated HDAC11 in AC-16 cardiomyocytes, with an early increase followed by a later decline, suggesting involvement in the acute metabolic stress response. Under diabetic cardiotoxic conditions, high glucose plus doxorubicin selectively increased the canonical HDAC11 isoform, whereas alternative isoform 3 was reduced, indicating isoform-specific regulation during heart failure–like injury. HDAC11 appeared predominantly cytosolic under basal conditions, while cardiotoxic stress suggested possible nuclear relocalization and transcriptional reprogramming. Intracellular miRNA profiling identified miR-145-5p as a putative upstream regulator of HDAC11.

CONCLUSIONI

These findings identify HDAC11 as an early epigenetic responder to metabolic and cardiotoxic stress. Its isoform-specific regulation, stress-dependent relocalization, and possible modulation by miR-145-5p may define an epigenetic–post-transcriptional axis in diabetic heart failure, suggesting HDAC11 as a potential biomarker and therapeutic target. Future in vivo validation in sham, transverse aortic constriction, and doxorubicin-induced cardiotoxicity mouse models, potentially combined with diabetic/metabolic stress, will clarify HDAC11 involvement in cardiac remodeling, cardiotoxic injury, and heart failure.

A061

INTEGRATED GENOMIC AND TRANSCRIPTOMIC PROFILING REVEALS THERAPEUTIC VULNERABILITIES IN METAPLASTIC BREAST CARCINOMA

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INTRODUZIONE

Metaplastic breast carcinoma (MBC) remains refractory to conventional chemotherapy with limited targeted therapeutic options. We aimed to characterize the genomic and pathway-specific landscape underlying distinct MBC subtypes and to identify potentially actionable therapeutic vulnerabilities.

METODI

TruSight RNA Pan-Cancer Panel (Illumina) was performed to identify novel transcripts, somatic variants, transcript variants and fusions in 7 squamous (SqC), 6 matrix-producing (Mp) and 10 spindle cells (SpC) MBCs compared with 12 triple-negative invasive ductal carcinomas of no special type (TNBC-NSTs) from our institutional cohort. Genomic and transcriptomic analyses were performed with OpenCravat and DESeq2. Statistical significance was set at $p < 0.05$. Funding: PNRR THE Spoke 6. CUP I53C22000780001.

RISULTATI

All 23 MBCs harbored recurrent somatic alterations in TP53 and chromatin remodeling genes, including KMT2C, EP300 and ARID1A. Compared to TNBC-NSTs, MBCs showed enrichment of alterations involving RTK-RAS, PI3K/AKT/mTOR, Hippo/Wnt, TGFß and NOTCH signaling pathways, alongside subtype-specific molecular profiles. SpC MBCs were enriched in NOTCH, PI3K, JAK1 and Hippo/Wnt pathway alterations, whereas SqC MBCs more frequently harbored EGFR, TP53 and RTK-RAS alterations. Mp MBCs showed alterations involving metabolic and epigenetic regulators, including ACACA, together with DNA damage response (DDR)-related pathways. SqC and SpC MBCs displayed higher tumor mutational burden than NSTs and Mp MBCs, alongside stem-like, EMT-high, migratory and angiogenic programs, consistent with a more aggressive and potentially immunogenic phenotype. In contrast, TNBC-NSTs showed a simpler TP53- and PDE4DIP-driven pattern. Clinically actionable alterations involving EGFR, ATM, PI3K/PTEN and chromatin-remodeling pathways were identified. Notably, RP1-34H18.1-NAV3 gene fusion (68%) was detected across all MBC subtypes.

CONCLUSIONI

MBC is a genetically complex and molecularly heterogeneous disease, distinct from TNBC-NST and characterized by subtype-specific genomic and transcriptomic differences. Our findings support tailored therapeutic strategies targeting EGFR/PI3K, Hippo/Wnt-EMT, immune and epigenetic/DDR pathways. Recurrent fusion transcripts previously unreported in MBC may have therapeutic relevance.

A062

ADDITIVE EFFECT OF RUTA GRAVEOLENS BIOACTIVE PHYTOCONSTITUENTS AND CISPLATIN THROUGH PKC/MEK/ERK PATHWAY IN GLIOBLASTOMA.

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INTRODUZIONE

Glioblastoma multiforme (GBM) is a lethal malignancy with limited therapeutic options. Its aggressive progression and resistance to standard therapy necessitate the development of novel therapeutic strategies. Plant-based compounds have emerged as promising candidates for therapeutic development modulating key targets in cancer. The Ruta graveolens displays anti-inflammatory, analgesic and antimicrobial properties. Herein, we report the anticancer potential of Ruta graveolens water extract (RGWE) on long-term cultures of human glioblastoma and elucidate the regulatory mechanisms driving its effect.

METODI

Secondary stabilized cell cultures (U-87 MG, T98MG, U-138 MG) and primary cell culture (FCN, MZC, GL18-15) were either treated with RGWE and/or cis-diamminedichloroplatinum (cisplatin, CDDP). Cytostatic responses were assessed by cell viability (TB exclusion test), cell cycle (PI staining) and apoptosis (Annex-V and Caspase-3) assays

RISULTATI

We demonstrate that RGWE slows glioblastoma growth without affecting apoptosis and arrests the cell cycle in the G2/M phase. Mechanistically, RGWE interferes with PKC/MEK/ERK signalling pathway, as assessed by Western Blot analysis, through PKC inhibition in its active form. Furthermore, the combination of RGWE and cisplatin enables the use of a sublethal dose of the latter (0.2 µg/ml), thereby reducing cytotoxicity and the resistance to the drug.

CONCLUSIONI

These findings reveal a novel mechanism by which RGWE controls glioblastoma growth, highlighting the therapeutic potential of targeting the PKC/MEK/ERK axis in glioma treatment.

A063

EXPLORING ASH1L–BRPF1 CHROMATIN CO-DEPENDENCIES TO IMPAIR ERα SIGNALING IN ENDOCRINE THERAPY RESISTANT BREAST CANCER

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INTRODUZIONE

Endocrine therapy resistance remains a major clinical challenge in estrogen receptor alpha–positive (ERα+) breast cancer (BC), where ongoing ERα activity is sustained by chromatin-associated regulatory mechanisms. This study aimed to identify and therapeutically exploit epigenetic dependencies that support resistant disease.

METODI

DepMap-derived co-essentiality analyses were integrated with functional validation in endocrine-sensitive and endocrine-resistant luminal BC models. BRPF1 and ASH1L were investigated by pharmacological inhibition, gene-targeting approaches and BRPF1-targeted proteolysis-targeting chimera (PROTAC)-mediated degradation. Effects on cell proliferation, apoptosis, estrogen-responsive transcriptional programs and tumor cell fitness pathways were assessed using cellular assays and transcriptomic/epigenomic profiling.

RISULTATI

BRPF1 emerged as a critical epigenetic dependency whose suppression impaired ERα+ BC growth across endocrine-sensitive, resistant, and patient-derived xenograft models. Network-based analyses revealed ASH1L as a major functional co-dependency of BRPF1. ASH1L pharmacological inhibition impaired estrogen-driven signaling, reduced proliferation, promoted apoptotic cell death, and compromised DNA damage repair processes. In ERα+ BC patient-derived organoids, ASH1L inhibition reduced cell viability and reshaped cancer-related transcriptional programs. Dual BRPF1/ASH1L inhibition achieved synergistic suppression of proliferation and disrupted ER-dependent transcriptional activity in endocrine-resistant models. Moreover, preliminary BRPF1-targeted PROTAC data suggest that protein degradation recapitulates and enhances BRPF1 inhibition, while retaining combinatorial activity with ASH1L blockade.

CONCLUSIONI

These findings define the BRPF1–ASH1L axis as a druggable epigenetic vulnerability in ERα+ breast cancer. Dual targeting of this circuitry, including BRPF1 degradation strategies combined with ASH1L inhibition, may provide a therapeutic approach to counteract endocrine therapy resistance.

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A064

DYNAMIC MODULATION OF HISTONE LACTYLATION IN PANCREATIC CANCER CELLS UNDER METABOLIC, EPIGENETIC AND THERAPEUTIC STRESS

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INTRODUZIONE

Histone lactylation is an emerging epigenetic modification linking metabolism to chromatin regulation. Pancreatic ductal adenocarcinoma (PDAC) is characterized by profound metabolic plasticity, but the dynamic regulation of histone lactylation under stress conditions remains poorly understood.

METODI

PL45 and MiaPaCa-2 pancreatic cancer cells were treated with sodium oxamate, SAHA, 5-FU, gemcitabine, and A-485 in time-course experiments (30 min–48 h). Site-specific histone lactylation and acetylation on H4K5, H4K8, H3K14, and H3K18 residues were evaluated by Western blot. Preliminary anti–lactyl-lysine immunoprecipitation coupled with mass spectrometry, STRING network analysis, and immunofluorescence assays were also performed.

RISULTATI

Histone lactylation showed rapid and treatment-dependent modulation in both PDAC cell lines. Oxamate induced transient lactylation reduction followed by partial recovery, whereas SAHA, 5-FU, and gemcitabine produced distinct temporal patterns across histone residues. Lactylation and acetylation displayed different kinetics, suggesting partially independent regulation. Among all treatments, A-485 induced the strongest reduction in histone lactylation levels. Preliminary proteomic analyses identified multiple putative lactylated proteins, including CNOT1 and EIF4G1, which were associated with pathways involved in miRNA-mediated post-transcriptional gene silencing. Immunofluorescence revealed nuclear lactylation signal consistent with the chromatin-associated nature of this modification.

CONCLUSIONI

Our findings demonstrate that histone lactylation is a highly dynamic and stress-responsive epigenetic mark in PDAC cells. The distinct temporal behavior observed between lactylation and acetylation suggests that lactylation may represent a previously underexplored adaptive chromatin response to metabolic and therapeutic stress. These data identify histone lactylation as a novel layer of epigenetic regulation in pancreatic cancer biology and support its potential relevance in metabolism-driven chromatin remodeling.

A065

LAMOTRIGINE AS A POTENTIAL THERAPEUTIC STRATEGY FOR BREAST CANCER: INVOLVEMENT OF THE FOXO3A/TXNIP AXIS.

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INTRODUZIONE

Resistance to endocrine therapy is a major clinical challenge in estrogen receptor #-positive (ER+) breast cancer (BC), underscoring the need for novel therapeutic strategies. Lamotrigine (LTG), an antiepileptic drug previously reported to enhance FoxO3a expression in Tamoxifen-sensitive and resistant ER+ BC cells, has emerged as a potential repurposing candidate. Since Thioredoxin-Interacting Protein (TXNIP) is a downstream target of FoxO3a, this study investigated whether LTG exerts its antitumor activity by modulating the FoxO3a/TXNIP pathway.

METODI

Experiments were performed using ER+ MCF-7 cells, tamoxifen-resistant cells (TamR), and patient-derived metastatic BC cells. RT-qPCR, Western blotting, ChIP assays, and chip-seq analyses were used to assess gene and protein expression and FoxO3a chromatin occupancy. Cell proliferation, migration, invasion, and spheroid growth were evaluated through Trypan Blue exclusion assay, Boyden chamber assays, and 3D spheroids. FoxO3a and TXNIP roles were investigated using siRNA-mediated silencing.

RISULTATI

ChIP-sequencing analysis performed in MCF-7 and TamR identified TXNIP as a common FoxO3a target gene. This finding was validated by ChIP assays, confirming FoxO3a recruitment to the TXNIP promoter and demonstrating that FoxO3a overexpression induces TXNIP expression. Consistently, following LTG exposure, FoxO3a recruitment to the TXNIP promoter was observed, accompanied by increased TXNIP expression and ROS levels in both cell lines. TXNIP silencing partially reversed the LTG-induced inhibition of cell proliferation, while silencing of either FoxO3a or TXNIP significantly attenuated LTG-mediated inhibition of proliferation and migration; combined silencing produced an even stronger reversal, particularly in TamR cells. LTG also reduced spheroid growth in both models. Importantly, LTG inhibited proliferation and migration while inducing expression of FoxO3a and TXNIP in patient-derived metastatic BC cells.

CONCLUSIONI

Our findings demonstrate that LTG exerts antitumor effects in ER+ BC by activating the FoxO3a/TXNIP axis, supporting the potential repurposing of LTG as a therapeutic strategy for BC, particularly for patients resistant to conventional hormonal therapies.
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A066

ROLE OF FOXO3A AND ANDROGEN RECEPTOR (AR) IN TRIPLE-NEGATIVE BREAST CANCER

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INTRODUZIONE

Triple-negative breast cancer (TNBC) is one of the most aggressive BC subtypes and still lacks effective targeted therapies. Therefore, identifying of novel molecular targets is crucial. FoxO3a is widely recognized as a tumor suppressor in several cancers, although its role in TNBC remains controversial and context-dependent. This study investigated the roles of FoxO3a and AR in TNBC by evaluating their expression, interactions, and biological and clinical impacts.

METODI

MDA-MB-231 and MDA-MB-468 BC cell lines were used. Protein expression levels and functional effects were evaluated by Western blotting, Trypan blue exclusion assay, and Boyden chamber migration and invasion assays. Protein interaction was analyzed by co-immunoprecipitation. In silico analyses were performed using cBioPortal, and prognostic significance was evaluated using the Kaplan–Meier Plotter.

RISULTATI

In silico analyses showed reduced FoxO3a and AR expression in TNBC compared with luminal subtypes, suggesting a functional correlation between the two molecules. Consistently, in vitro experiments demonstrated that individual overexpression of FoxO3a or AR did not significantly affect proliferation, migration, or invasion. Conversely, co-expression of both proteins significantly reduced proliferation and markedly inhibited migration and invasion, suggesting a potential cooperative tumor-suppressor effect in TNBC. Co-immunoprecipitation confirmed a physical interaction between FoxO3a and AR. Moreover, Kaplan–Meier Plotter analysis revealed that their co-expression was associated with a favorable prognosis in TNBC patients, particularly in tumors with high AR expression, suggesting that FoxO3a may enhance the favorable prognostic role of AR.

CONCLUSIONI

These findings suggest that AR acts as a functional cofactor of FoxO3a in TNBC. Therefore, combined modulation of FoxO3a and AR may represent a promising therapeutic strategy for TNBC and support the development of novel targeted approaches.
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A067

ICOSL REVERSE SIGNALING DRIVES IMMUNE SUPPRESSION AND HEPATOCARCINOGENESIS IN MASH-HCC

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INTRODUZIONE

Metabolic-associated steatohepatitis (MASH) can progress to hepatocellular carcinoma (MASH-HCC), a major cause of primary liver cancer worldwide. Since chronic inflammation sustained by macrophage and T-cell interactions plays a central role in MASH-HCC pathogenesis, we investigated the contribution of the ICOS/ICOSL co-stimulatory pathway in a murine model of MASH-related hepatocarcinogenesis. Besides acting as the ligand for ICOS, ICOSL can also transduce intracellular signals in antigen-presenting cells through a mechanism known as reverse signaling, potentially modulating inflammatory and immune responses.

METODI

Wild-type (WT), ICOS- and ICOSL-knockout (ICOS/ICOSL-KO) mice were subjected to DEN injection followed by CDAA diet feeding. A subset of WT, ICOS-KO, and ICOSL-KO mice received recombinant soluble ICOS (ICOS-Fc) during the final 8 weeks of treatment to boost ICOSL reverse signaling. In parallel, in vitro experiments were performed in RAW264.7 macrophages stimulated with ICOS-Fc, and conditioned media were subsequently used to stimulate Hepa1-6 hepatocarcinoma cells.

RISULTATI

Both ICOS- and ICOSL-KO mice displayed reduced tumor burden, with fewer and smaller HCC nodules compared to WT animals. Tumor protection was associated with reduced neoangiogenesis, shown by lower CD31 expression, and increased formation of CD8+ T cell-enriched tertiary lymphoid structure (TLS)-like aggregates, consistent with enhanced anti-tumor immune surveillance in ICOS/ICOSL-deficient mice. Moreover, ICOS/ICOSL deficiency was associated with reduced activation of the IL-6/STAT-3 pathway. Conversely, boosting ICOSL reverse signaling through administration of recombinant ICOS-Fc markedly worsened tumor progression in WT and ICOS-KO mice, while showing no effects in ICOSL-KO animals. ICOS-Fc enhanced neoangiogenesis and tumor cell proliferation, reduced TLS formation and CD8+ T-cell accumulation and established an immunosuppressive microenvironment. In vitro, ICOS-Fc stimulated macrophage IL-6 production, and conditioned media from ICOS-Fc-treated RAW264.7 promoted Hepa1-6 cell proliferation. Furthermore, ICOS-Fc induced macrophage Annexin A1 production both in vitro and in vivo, supporting the establishment of an anti-inflammatory and immunosuppressive microenvironment favoring tumor progression.

CONCLUSIONI

Overall, these findings identify the ICOS/ICOSL axis as a critical regulator of immune surveillance, angiogenesis, and tumor progression in MASH-HCC, supporting its potential as a novel therapeutic target.

A068

CONTRIBUTION OF MITOCHONDRIAL DNA TO THE ONSET AND MAINTENANCE OF INFLAMMATION IN PATIENTS WITH SYSTEMIC SCLEROSIS: FOCUS ON CARDIOVASCULAR COMPLICATIONS

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INTRODUZIONE

Systemic sclerosis (SSc) is a chronic inflammatory autoimmune disease characterized by microvascular damage, skin fibrosis and immune system defects. Circulating free mitochondrial DNA (cf-mtDNA) may play a role in disease progression by acting as a damage-associated molecular pattern (DAMP) that promotes the activation of innate immune cells. Cf-mtDNA is sensed by monocytes via TLR9, inducing the expression of IL-6, TNFα, and IFNα, while NLRP3 inflammasome senses ox-mtDNA and promotes the maturation of IL-1β and IL-18. However, it remains unclear whether the pro-inflammatory potential of mtDNA changes during disease progression and contributes to immune dysregulation.

METODI

70 SSc patients (mean age 60 # 14) along with 67 age- and sex-matched healthy controls (HC) were enrolled. Cf-mtDNA was extracted from plasma samples, and ddPCR assay was used to quantify cf-mtDNA. Monocytes were isolated from a subset of patients and treated with mtDNA alone or in combination with LPS, to evaluate metabolic features of monocytes. Pro-inflammatory cytokines were quantified to assess mtDNA capability to activate a pro-inflammatory response.

RISULTATI

Plasma cf-mtDNA levels were lower in SSc patients than in HC. Monocytes from SSc patients showed reduced basal respiration under all conditions, indicating impaired metabolic activity. Maximal respiration increased after LPS stimulation, alone or combined with mtDNA, in both groups. However, only SSc monocytes exhibited an enhanced response to mtDNA, with increased non-mitochondrial respiration. Pro-inflammatory cytokine levels were higher in SSc patients in resting condition. LPS increased TNF-α, IL-1β, IL-6, and IL-8 expression, with no additional effect from mtDNA co-stimulation. mtDNA alone induced IL-1β and IL-6 expression in both groups, with a huge effect in SSc patients, particularly for IL-1β.

CONCLUSIONI

Despite reduced plasma mtDNA levels in SSc patients, monocytes from SSc patients showed altered immunometabolic features and increased responsiveness to mtDNA.

A069

VALIDATION OF PATIENT-DERIVED LIVER ORGANOIDS FOR IN VITRO HEPATIC STUDIES AND TESTING OF ACTIVE AGENTS

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INTRODUZIONE

Liver diseases represent a global health challenge, and the increasing prevalence of metabolic disorders is set to exacerbate this crisis. To meet evolving regulatory demands, patient-specific in vitro liver models are essential for understanding disease mechanisms and developing new therapeutic strategies. Organoids models, which faithfully recapitulate liver biology, can be established from both tumor and non-tumor liver tissues, offering insight into various liver conditions, from acute injuries to chronic diseases.
The aim of this study was to develop patient-derived liver organoids to improve the understanding of liver diseases, facilitating advancements in personalized medicine.

METODI

To developed organoids, we minced non-tumor biopsies and shortly digested into small cell clusters which were then embedded in Matrigel domes and cultured in growth factors-enriched medium. Subsequently, liver organoids were treated with the natural stilbene resveratrol (RSV), and its analogue 4, 4'-dihydroxy-trans-stilbene (DHS) for 48h, measuring cell viability, and their metabolism, analyzing their supernatants by HPLC-MS. In addition, after oleic acid stimulation, liver organoids were treated with 2-methyl-6-(phenylethynyl) pyridine (MPEP), a mGluR5 inhibitor evaluating the lipid content.

RISULTATI

We developed and characterized liver organoids verifying the presence of specific markers. Subsequently, based on a preliminary morphological and functional analysis, we show a slight change in metabolites present after treatments with RSV and its analogue DHS. In parallel, to in vitro represent steatosis pathology, we treated non-tumor organoids with oleic acid and test the effect of MPEP, that reduced lipid accumulation, acting on AMPK pathway, confirming previous data showed on bidimensional HepG2. In both bidimensional and three-dimensional non-steatotic models, dihydroxyphenylglycine (DHPG), a mGluR5 agonist, slightly increased lipid accumulation, an effect reversed by co-treatment with MPEP, confirming mGluR5 involvement.

CONCLUSIONI

We initially demonstrated that patient-derived liver organoids represent a reliable three-dimensional in vitro model to study the effects of different compounds at hepatic level.

A070

UNRAVELLING THE ROLE OF THE MITOCHONDRIAL DEACETYLASE SIRT3 IN GASTRULOID DEVELOPMENT

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INTRODUZIONE

Gastrulation is a critical stage of early mammalian development, highly sensitive to perturbations leading to embryonic abnormalities and impaired tissue organization. Stem cell–based embryo models such as gastruloids provide a powerful in vitro system to study early developmental processes. Within this context, energy metabolism has emerged as a regulator of cell fate specification and tissue patterning, although its role during early embryogenesis remains poorly understood. Supporting this concept, a previous pharmacological screening of Epi-drugs identified Sirtuin signaling as a potential regulator of gastruloid development, as the pan-Sirtuin inhibitor UVI5062 altered gastruloid morphology and patterning. Building on these observations, the present study focused on Sirt3, a mitochondrial NAD +-dependent deacetylase.

METODI

Mouse embryonic stem cell–derived gastruloids were used as an in vitro model. The effects of Sirt3 perturbation on gastruloid development were evaluated through morphological analysis by bright-field imaging to assess changes in elongation and structural organization, combined with immunofluorescence staining for lineage-specific and tissue-patterning markers, as well as gene expression analyses to characterize alterations in developmental programs and cell fate specification.

RISULTATI

Single-cell transcriptomic analysis showed that Sirt3 expression peaks during the developmental window immediately following CHIR-mediated WNT activation. Both pharmacological inhibition of Sirt3 (3-Typ) and Sirt3 silencing disrupted gastruloid development, inducing aberrant morphologies and axial defects. These abnormalities were associated with expansion of the posterior mesodermal marker Bra and of Sox1/Sox2-positive neuronal domains, delineating an epithelial-like structure absent in controls.

CONCLUSIONI

These findings suggest that mitochondrial Sirt3 signaling is essential for proper embryonic patterning and lineage. Its disruption induces developmental abnormalities, highlighting mitochondrial regulation as a contributor to developmental disease mechanisms. Ongoing single-cell RNA sequencing and mitochondrial functional analyses will further clarify its role, while validation in human gastruloids will assess the relevance of these findings to human development and disease.

A071

THE MEMORY TEST: PERSONALIZING SARS-COV-2 BOOSTER VACCINATIONS

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INTRODUZIONE

Extensive SARS-CoV-2 vaccination programs have significantly reduced viral spread. However, heterogeneous vaccine efficacy often necessitates booster doses. Current fixed-timeline booster strategies can lead to unnecessary vaccinations, burdening healthcare systems. Identifying reliable immunological profiles that predict protection against re-infection is crucial for developing personalized vaccination strategies based on an individual's actual immune status. The primary aim of this study was to develop and validate the "Memory Test," a novel two-step in vitro assay designed to comprehensively assess protective immune memory against SARS-CoV-2.

METODI

We developed and validated a novel two-step assay, the "Memory Test," to assess protective immune memory. Peripheral blood mononuclear cells (PBMCs) from vaccinated (SV), unexposed (SU), and Rituximab-treated vaccinated (SVR) subjects were analyzed. In Step 1, plasma neutralizing activity (NA) was measured. PBMCs were then stimulated with inactivated SARS-CoV-2 (iSARS) for 5 days. Secretomes were analyzed via multiplex ELISA, and T/B cell subpopulations via flow cytometry. In Step 2, Step 1 supernatants and PBMCs were cultured with SARS-CoV-2-infected A549-ACE2 cells to evaluate the control of viral replication.

RISULTATI

Inhibition of SARS-CoV-2 infection in SV subjects did not significantly correlate with NA titers. However, iSARS-stimulation markedly decreased viral load in SV subjects. Antiviral responses were strongly B-cell dependent, demonstrating remarkably higher protection in iSARS-stimulated PBMCs from SV compared to immunocompromised SVR subjects. While T cell subpopulations showed no meaningful alterations upon stimulation, secretome analysis revealed that SV subjects produced significantly higher levels of protective cytokines (IFN-γ, IL-4, IL-8, MCP-1) than SVR individuals.

CONCLUSIONI

Neutralizing antibody titers alone are insufficient to predict comprehensive protective immunity. The "Memory Test" provides a reliable, practical tool to evaluate cellular and humoral protective immune memory. This assay can inform evidence-based, personalized decisions regarding the necessity of booster doses, offering particular clinical value for immunocompromised patients.

A072

COX-2 INHIBITION ATTENUATES GLYCATION STRESS, INFLAMMATION AND SENESCENCE IN TEMOZOLOMIDE-TREATED GLIOBLASTOMA CELLS

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INTRODUZIONE

Glioblastoma (GBM) is characterized by marked resistance to Temozolomide (TMZ), driven by adaptive mechanisms including metabolic rewiring, inflammation, glycation stress, and therapy-induced senescence. Increasing evidence suggests that TMZ paradoxically promotes pro-tumorigenic responses that sustain tumor survival and therapeutic failure. In this study, we investigated the role of cyclooxygenase-2 (COX-2) in TMZ-induced metabolic and senescence-associated adaptations and evaluated whether its selective inhibition by celecoxib (CXB) could counteract these resistance-associated mechanisms.

METODI

TMZ-sensitive and -resistant GBM models, including established cell lines and patient-derived primary culture, were treated with TMZ, CXB, or their combination. Metabolic, glycative, inflammatory, and senescence-associated responses were assessed using Seahorse metabolic analysis, Western blotting, flow cytometry, ELISA, immunofluorescence, ß-galactosidase (ß-gal) staining, and real-time PCR.

RISULTATI

TMZ induced metabolic rewiring characterized by increased c-Myc and GLUT-1 expression, enhanced glycolytic ATP production, glycation stress, AGE/RAGE signaling, NF-κB activation, inflammatory cytokine release, and senescence-associated features. These adaptive responses were particularly evident in MGMT-deficient and p53 wild-type cells. CXB cotreatment significantly counteracted TMZ-induced glycolysis, reduced intracellular AGE accumulation, suppressed RAGE/NF-κB/NOS2 signaling, and attenuated SASP-associated cytokine. Moreover, CXB reduced TMZ-induced senescence markers, including ß-gal positivity and p53/p21 upregulation. Despite marked heterogeneity among GBM models, COX-2 inhibition consistently disrupted resistance-associated adaptive responses.

CONCLUSIONI

Our findings identify a COX-2-driven glyco-inflammatory-senescence axis as a central adaptive mechanism underlying TMZ resistance in GBM. COX-2 inhibition by CXB hampers this protumorigenic loop, restoring TMZ sensitivity by targeting metabolic rewiring, glycation-associated stress, inflammation, and senescence. These results support the further preclinical evaluation of COX-2-targeting strategies as adjuvant approaches for overcoming therapy resistance in GBM.

A073

EXPLORING THE CONTRIBUTION OF FINEGOLDIA MAGNA IN OBESITY-RELATED COLORECTAL CARCINOGENESIS.

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INTRODUZIONE

Obesity and gut microbiota dysbiosis are increasingly recognised as important contributors to colorectal cancer (CRC) development, although the role of specific pathobionts in obesity-associated tumorigenesis remains poorly understood. In a previous study analysing mucosa-associated microbiota (MAM) from colorectal polyps of 120 patients, we identified an enrichment of Finegoldia magna in overweight/obese subjects compared to normal-weight ones. Due to its virulence factors involved in epithelial adhesion and tissue invasion, F. magna may contribute to colorectal carcinogenesis.

METODI

The effects of F. magna were investigated using integrated in-vitro and ex-vivo models. Caco-2 intestinal epithelial cells were exposed to live or heat-inactivated F. magna to assess bacterial adhesion by Gram staining, and gene expression alterations by RNA-Sequencing. Intestinal barrier integrity was evaluated using an ex-vivo mouse colon perfusion system, where FITC-dextran permeability and expression of tight junction genes, including OCLN, were analysed following exposure to live bacteria.

RISULTATI

Live F. magna, but not heat-inactivated bacteria, adhered strongly to Caco-2 epithelial cells and induced a pro-inflammatory response, highlighting the importance of bacterial viability in host interaction. Increased expression of neutrophil-recruiting chemokines, including CXCL8 and CXCL5, was observed together with activation of chemokine signalling pathways.
In the ex-vivo colon perfusion model, exposure to live F. magna significantly increased FITC-dextran permeability, indicating disruption of epithelial barrier integrity. In parallel, qRT-PCR analysis revealed increased OCLN expression, suggesting a compensatory response to bacterial-induced epithelial stress.

CONCLUSIONI

Overall, our findings suggest that F. magna may act as a pathobiont that promotes inflammation and impairs epithelial barrier integrity, potentially contributing to obesity-associated colorectal tumorigenesis. Future in-vivo studies using CDX2-Cre/Apc^fl/+^ mice under standard or Western diet conditions will further investigate the combined effects of obesity and F. magna colonisation on CRC development.
This project is supported by AIRC-25886.

A074

ROLE OF 5-ALA-PDT IN MODULATING PROLIFERATION, APOPTOSIS AND ROS PRODUCTION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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INTRODUZIONE

Head and neck carcinoma (HNC) is one of the most common types of cancer worldwide and it is a leading cause of deaths. Despite advances in diagnosis and treatment, long-term survival has improved only slightly in recent years. 5-aminolevulinic acid (5-ALA)-mediated photodynamic therapy (PDT) leads to the accumulation of protoporphyrin IX (PpIX), a molecule of the heme biosynthetic pathway. PDT, based on the reaction between light-activated PpIX and molecular oxygen, leads to cell death due to an increase in intracellular reactive oxygen species (ROS). This study analyses the effect of ALA-PDT on HNC cell lines and tissues.

METODI

CAL-27 and OECM-1 cell lines were treated with low doses of 5ALA for 8h and 12h, respectively, followed by irradiation with a 630 nm LED light source to induce PDT. Cytotoxicity was evaluated using the MTS assay. Gene expression of apoptosis and ROS was analysed by qRT-PCR. The same experimental approach was applied on HNC patient tissues. Cancer tissues were sectioned using a vibratome through the precision-cut slices (PSC) technique and then analysed for the same apoptotic and ROS pathways in order to validate the in vitro findings in a translational model.

RISULTATI

The highest cell mortality was observed in CAL-27 cells at 1.84 mM for 8h, while OECM-1 cells showed the greatest effect at 0.92 mM after 12h. qPCR analysis revealed an upregulation of BAX and p21, suggesting a shift in the cellular balance toward programmed cell death. Oxidative stress analysis showed increased SOD and CAT expression, together with a more marked upregulation of iNOS, indicating enhanced ROS production and activation of antioxidant defences that were insufficient to counteract oxidative damage. Similar molecular changes were observed in ex-vivo tissue samples, confirming the in vitro findings.

CONCLUSIONI

ALA-PDT markedly inhibited the viability of HNC cell lines through mechanisms involving the accumulation of PpIX that, under irradiation, can induce profound ROS production and phototoxicity. This could support the development of new therapeutic strategies for HNC and represent an alternative to conventional therapies for this cancer, such as radiotherapy, significantly increasing the quality of life of patients who often present side effects such as xerostomia.

A075

EFFECTS OF THE PROBIOTIC MIXTURE SLAB51 ON THE GUT MICROBIOTA AND ON BRAIN AQP4EX EXPRESSION OF AGED C57BL/6J MICE

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INTRODUZIONE

OXXYSLAB (formerly SLAB51) is a multi-strain probiotic containing eight lyophilized lactic acid bacteria and bifidobacteria (S. thermophilus CNCM I-5570; L. brevis I-5566; L. plantarum I-5569; L. paracasei I-5568; L. acidophilus I-5567; L. helveticus I-5573; B. animalis subsp. lactis I-5571 and I-5572), with potential gut–brain axis activity. Aquaporin-4 (AQP4) is the main water channel in the brain, where it regulates water homeostasis and glymphatic clearance. We tested whether chronic exposure modulates gut microbiota, frailty, survival and brain AQP4ex in aged C57BL/6J mice.

METODI

Directly supplemented mice (SLAB51, n=40), cohoused untreated mice (CoH, n=39) and age-matched naïve controls (CTRL, n=26) were compared at ~29 months. All animals were C57BL/6J bred in-house. Clinical Frailty Index (CFI), survival and fecal microbiota were assessed. Analyses included pairwise/adjusted PERMANOVA, CLR-based differential abundance at genus level with FDR correction, species-level analysis on raw abundances, and a 3-class Random Forest with cross-validation. Brain AQP4ex was measured by western blot in a subset of SLAB51 and CoH mice at ~33 months.

RISULTATI

SLAB51-exposed males showed improved CFI versus controls at the third follow-up, with no survival benefit. Gut microbiota of exposed mice (SLAB51 and CoH) differed from CTRL (Random Forest AUC = 0.94, permutation $p < 0.001$; pairwise PERMANOVA $p < 0.05$), whereas SLAB51 and CoH were not separable (PERMANOVA $p = 0.88$; AUC ≈ 0.60, n.s.), indicating cohousing-mediated transfer. The genus Streptococcus showed the largest shift (prevalence 85–88% vs 27% in CTRL, $p < 10^{-6}$), driven by S. danieliae; the probiotic-derived S. salivarius subsp. thermophilus was detected exclusively in exposed mice, albeit at low prevalence and abundance. Lactobacillus, Adlercreutzia and Stoquefichus were also higher in exposed mice, while Bifidobacterium and Parabacteroides were unchanged after correction. Brain AQP4ex was higher in SLAB51 than CoH mice.

CONCLUSIONI

Chronic SLAB51 exposure was associated with a reproducible gut signature transferable through cohousing, detection of the probiotic-derived S. thermophilus, improved male frailty and higher brain AQP4ex in directly supplemented mice, supporting further investigation as a modulator of the gut–brain axis in aging.

A076

INTRACELLULAR LIGAND-INDEPENDENT TRKA ACTIVATION PROTECTS SH-SY5Y NEUROBLASTOMA CELLS AGAINST COPPER CYTOTOXICITY

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INTRODUZIONE

Copper, by changing oxidation states within cells, induces cell death through either cuproptosis, by binding to lipoylated proteins in the TCA cycle, apoptosis by generating excessive ROS, or by a mixture of the two. Copper cytotoxicity of relevance to neurodegenerative diseases and is also being explored as a potential therapeutic treatment for neuroblastoma (NB), which due to high metabolic activity are distinctly vulnerable to copper-induced cytotoxicity. Considering our interest in how NB cells escape programmed cell death mechanisms, we investigated the relative sensitivity of SH-SY5Y NB cells and transfected counterparts engineered to express full length TrkA or its oncogenic TrkAIII isoform, to copper cytotoxicity, with the aim of identifying potential evasion mechanisms.

METODI

Stable SH-SY5Y pcDNA, TrkA and TrkAIII transfectants were treated with CuSO4. Total and phosphorylated TrkA/TrkAIII expression was assessed by Western blotting after 1, 6 and 24 hours of treatment, in the presence or absence of entrectinib. Cytotoxicity was monitored by live-cell imaging using the IncuCyte® S3 system and Cytotox Green dead-cell stain in the presence or absence of entrectinib, z-VAD-fmk or necrostatin-1.

RISULTATI

Live-cell imaging confirmed marked CuSO4 cytotoxicity in pcDNA SH-SY5Y cells, substantially reduced by z-VAD-fmk but not by necrostatin-1. TrkA transfectants exhibited high resistance, whereas TrkAIII transfectants showed intermediate resistance to CuSO4 cytotoxicity. Resistance in both TrkA and TrkAIII transfectants was reduced by entrectinib. In TrkA transfectants, resistance was associated with gradual conversion of mature cell surface gp140 TrkA to an activated intracellular immature gp110 isoform. In contrast, TrkAIII transfectants exhibited no CuSO4-induced changes in TrkAIII expression or activation.

CONCLUSIONI

Preliminary data indicate that CuSO4 cytotoxicity in SH-SY5Y cells occurs primarily through apoptosis rather than necroptosis. TrkA and TrkAIII expression confer different levels of resistance to copper cytotoxicity. Resistance in TrkA transfectants appears associated with intracellular accumulation and activation of immature gp110 TrkA, whereas TrkAIII resistance depends on constitutive TrkAIII activity.

A077

ALTERNATIVE NF-YA SPLICING REGULATES TUMORIGENICITY, SENSITIVITY TO DOXORUBICIN AND PHENOTYPE CONVERSION IN NON-MYCN AMPLIFIED SH-SY5Y NEUROBLASTOMA CELLS

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INTRODUZIONE

Neuroblastomas (NB) are aggressive tumors that exhibit high intra-tumoral heterogeneity and dynamic phenotypic plasticity despite low mutational burden. Interconversion between adrenergic and mesenchymal states, regulated by developmental, microenvironmental and therapeutic cues, result in therapy-induced mesenchymal conversion that promotes therapy resistance, post therapeutic relapse, progression and poor clinical outcome. The NF-Y transcription factor is involved in phenotypic plasticity through alternative NF-YAI and NF-YAs splicing. The novel NF-YAx isoform is induced by doxorubicin (Dox) in NB cells, suggests roles in phenotype plasticity, resistance and disease progression. Here we investigate how NF-YA isoforms associate with phenotype heterogeneity in an SH-SY5Y model of non-MYCN amplified NB.

METODI

RT-PCR of key adrenergic and mesenchymal/neural-crest markers in stable control, NF-YAI, NF-YAs and NF-YAx SH-SY5Y transfectants as potential predictors of phenotypes and analysis of resistance to doxorubicin in cytotoxicity assays.

RISULTATI

Control transfectants (mixed NF-YAI/NF-YAs) are tumorigenic, sensitive to doxorubicin and display high GATA3, TOP2B, NOTCH3 and low CD44/undetectable PRRX1, suggesting a proliferating adrenergic, less stem-like state. NF-YAI transfectants are less tumorigenic, Dox-sensitive and display high GATA3, TOP2B, NOTCH3; low CD44; detectable PRRX1 expression suggesting an intermediate hybrid adrenergic/mesenchymal profile with low NCSC identity. NF-YAs transfectants are more tumorigenic lack GATA3 expression but express PRRX1, CD44, TOP2B and NOTCH3, suggesting more tumorigenic mesenchymal, NCSC state. NF-YAx transfectants are tumorigenic, Dox-resistant, display high GATA3, TOP2B, NOTCH3 and CD44 expression, suggesting a hybrid state with marked NCSC identify.

CONCLUSIONI

NF-YAI, NF-YAs and NF-YAx promote distinct transcriptional states in SH-SY5Y cells, confirming functional differences, NF-YAI moves cells towards a less-tumorigenic, Dox-sensitive more adrenergic state, NF-YAs moves cell towards a more tumorigenic Dox-sensitive less-differentiated mesenchymal state, whereas NF-YAx moves cells to a tumorigenic, Dox-resistant, hybrid mesenchymal/adrenergic phenotype with neural crest stem cell identity.

A078

HYPERGLYCEMIA REDUCES TYROSINE HYDROXYLASE EXPRESSION AND INCREASES G-QUADRUPLEXES FORMATION IN SH-SY5Y CELLS

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INTRODUZIONE

Cognitive impairment is a complication of Diabetes mellitus (DM). Dopamine (DA) homeostasis is crucial for cognitive function and is altered in DM. Animal studies suggest that chronic hyperglycemia contributes to brain dopaminergic dysfunction, but the underlying molecular mechanism are still unclear. We hypothesize that hyperglycemia could affect DA levels by altering the expression of genes involved in DA synthesis and degradation, including TH (Tyrosine Hydroxylase), DDC (DOPA decarboxylase), COMT (Catechol-O-methyltransferase), MAO (Monoamine oxidase),and α-Syn (α-synuclein). Evidence in literature and computational analysis evidenced the presence of putative G-quadruplexes (G4)-forming sequences (PQS) in the promoters of some of these genes. Since G4 are DNA secondary structures implicated in gene regulation and neurodegeneration, we investigated G4 role in hyperglycemia induced DA imbalance.

METODI

Human neuroblastoma SH-SY5Y cells were exposed to high glucose (HG,50mM) for different times (48–96h). Total G4 content was evaluated by flow cytometry using the G4-specific BG4 antibody. Protein and mRNA levels of COMT, MAO-B, α-Syn, TH and DDC were analysed by Western blot and RT qPCR.

RISULTATI

The evaluation of total G4 content revealed a progressive time-dependent significant increase of G4 stability in response to HG at 48,72 and 96h. Thus, we evaluated the protein levels of COMT, MAO-B, α-Syn, TH and DDC in SH-SY5Y cells exposed to HG for 48h. We found that HG treatment did not significantly modify the protein levels of COMT, MAO-B, α-Syn and DDC, but induced a decrease by 30% in the TH protein levels. TH mRNA levels were also reduced by almost 60%. In silico analysis of the human TH promoter using the pqsfinder algorithm identified multiple putative G4-forming sequences, including high-scoring putative quadruplex sequences (PQS). Overall, these findings support the presence of potential G4 regulatory elements within the TH promoter and provide a rationale for further investigating whether HG-induced G4 accumulation contributes to TH downregulation.

CONCLUSIONI

Our findings indicate that HG increases G4 formation and reduces TH expression in human neuronal cells. Further studies will be required to clarify the impact of these alterations on DA homeostasis and the causal role of G4.

A079

MODULATION OF REDOX BALANCE AND INDUCTION OF CELL DEATH BY TIRE WEAR PARTICLES POLLUTANTS

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INTRODUZIONE

Tire wear particles (TWPs), generated by tire abrasion during vehicle use, are emerging environmental contaminants widely dispersed in air, water, and soil. Human exposure may occur through ingestion and inhalation, raising concerns about their potential impact on human health. This study aimed to investigate the possible toxic effects of TWPs on intestinal epithelium, evaluating their impact on enterocyte-like cell redox balance and viability.

METODI

TWPs were tested on differentiated Caco-2 (human colorectal adenocarcinoma) cells at concentrations considered environmentally relevant and potentially achievable in vivo following human environmental exposure (0.5 - 5 μg/mL) and at different incubation times (24 – 72 h). Cell viability was evaluated via the MTT assay. Furthermore, the change in concentration of reduced glutathione (GSH) in relation to its oxidized form (GSSG) was measured via HPLC analysis coupled with an electrochemical detector. ROS production was detected through spectrophotometric H_2-DCF-DA assay. Nitric oxide (NO) production was also evaluated by Griess' reagent assay.

RISULTATI

Cytotoxicity emerged after 48 h, starting from low TWPs concentrations, while cell viability partially recovered after 72 h of treatment. TWPs exposure induced a concentration- and time-dependent effect on intestinal cells. Induction of NO release was observed only at the highest tested concentration (5 μg/mL). A decrease in the GSH/GSSG ratio was detected at all tested concentrations, except for 5 μg/mL, where the ratio increased compared to control cells, likely as a cellular adaptive response to oxidative stress. Dose-dependent ROS production was detected only after 24 h of exposure, whereas ROS levels returned to baseline at longer incubation times in parallel with the GSH level increase.

CONCLUSIONI

This study highlighted the toxic potential of TWPs on intestinal cells, particularly at higher concentrations, where modulation of GSH and NO levels increased oxidative stress, and partial impairment of cell viability was observed. These findings suggest that TWPs may contribute to intestinal cellular damage and support the need for further investigation into their potential health risks.

A080

FUNCTIONAL EFFECTS OF SELECTED SYBODIES TARGETING SERPINB3 IN HEPG2 CELLS

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INTRODUZIONE

Squamous cell carcinoma antigen 1 (SERPINB3) belongs to the serine protease inhibitor family, which is expressed in epithelial cells, leukocytes, and primary liver tumors. SERPINB3 mediates various processes, including the regulation of inflammatory response, metabolism, and cell death. Both autocrine and paracrine signaling pathways are affected by SRPINB3's inhibition of cysteine proteases. Because of its role in the development of cancer, and cellular defence against oxidative stress, it is important in both physiological and pathological contexts, which makes SERPINB3 an attractive therapeutic target.

METODI

To isolate nanobodies against SERPINB3, a hybrid selection strategy was employed, combining one round of ribosome display and two rounds of phage display. The enrichment efficiency was tracked by qPCR after each selection round, enabling real-time assessment and screening for selection pipeline problems. ELISA screening was performed to select high potential binders. AlphaFold predicted the structural models of the selected nanobodies, and their interactions with SERPINB3 were predicted by docking analysis using ClusPro (Antibody mode). These nanobodies were evaluated in HepG2 cells overexpressing SERPINB3 to assess their effects on cell proliferation, migration, invasion, and cytotoxicity.

RISULTATI

ELISA screening confirmed that seventeen sybody clones had more than 1.5-fold enrichment. Seven were selected based on their binding strength and sequence diversity. The nanobodies B3, B10, D5, G6, and G12 were predicted to interact with the Reactive Center Loop of SERPINB3. In contrast, the nanobody A12 was predicted to bind to α-helix A and G on SERPINB3, while H8 nanobody interacts with β-sheet A and α-helix G. All the nanobodies were successfully expressed in E.coli and purified for evaluation of their effects in HepG2 cells. Nanobodies A12, G6, B10, and D5 showed a promising inhibitory effect on cell proliferation. In addition, nanobody D5 significantly inhibited cell invasion. However, none of the nanobodies showed a significant effect on migration or cytotoxicity. Overall, the nanobodies showed selective effects on proliferation and invasion, with no significant impact on migration or cytotoxicity.

CONCLUSIONI

These sybodies may selectively modulate specific tumor-associated cellular processes, particularly proliferation and invasion, highlighting their potential as targeted therapeutic candidates against SERPINB3-related cancer phenotypes.

A081

MEBENDAZOLE AND NITROXOLINE IN COMBINATION SHOW EFFICACY IN CELL AND ANIMAL MODELS OF PANCREATIC CANCER

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INTRODUZIONE

Pancreatic cancer (PC) is one of the most challenging tumors, thus more effective and safer agents are needed. Drug repurposing represents a valuable strategy to exploit approved non-anticancer drugs in oncology. Mebendazole (MBZ) and nitroxoline (NTX) are promising repurposable drugs that exhibit antitumor properties as single agents in preclinical PC models. Here, we evaluated the antitumor potential of MBZ and NTX in combination (MBZ/NTX) using 2D and 3D spheroid PC models and explored their effectiveness in a mouse PC xenograft model.

METODI

Different PC cell lines were used to assess the antitumor effects of MBZ and NTX in combination. Viability and clonogenicity were evaluated by MTT and clonogenic assays. Flow cytometry and/or immunoblot analyses of PARP cleavage, cell cycle proteins, and EMT markers were used to evaluate apoptosis, cell cycle distribution and cell invasiveness. Cytoskeletal organization and Boyden chamber invasion assays were also performed. Two PC-derived 3D spheroids were generated to test the effects of treatments on spheroid size and death. L3.6pl cell-derived mouse xenografts were established to assess the effects of the two drugs on PC growth.

RISULTATI

MBZ/NTX markedly inhibited PC cell viability compared to single agents, with L3.6pl and PATU-8902 identified as the most sensitive cells. In these PC cell lines, MBZ/NTX impaired clonogenicity, promoted apoptosis, and altered cell cycle progression. Furthermore, the combination suppressed cell invasiveness and disrupted actin organization, partially reverting the mesenchymal phenotype toward an epithelial-like state. In 3D models, MBZ and NTX reduced spheroid growth and enhanced cell death. In line with 3D predictions, NTX, MBZ, and their combination reduced tumor growth in L3.6pl xenograft mouse models, with no toxicity across all regimens.

CONCLUSIONI

Our data show that MBZ and NTX in combination exert potent, cooperative antitumor activity in 2D, 3D, and in vivo models of PC, thus representing a promising therapeutic strategy to be exploited in PC treatment.

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A082

ZC3H13 SHAPES M6A TOPOLOGY TO GOVERN MYC AND MAPK3 EXPRESSION IN LEUKEMIA

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INTRODUZIONE

BACKGROUND AND AIMS:
N6-methyladenosine (m6A) RNA modification is a critical regulator of the RNA life cycle and gene expression. Because dysregulation is linked to malignancy, elucidating how the m6A machinery influences oncogenic programs is essential for new therapeutic discovery. While ZC3H13 is a known non-catalytic component of the m6A writer complex required for its organization and stability, its specific role in leukemogenesis has yet to be elucidated. Herein, we aim to explore ZC3H13 as a therapeutic target in leukemia.

METODI

METHODS:
ZC3H13 function in leukemia was investigated by using shRNA-lentiviral based knockdown approach across leukemic cell lines. Cellular phenotypes including proliferation, apoptosis, and cell cycle assays were assessed by FACS, qRT-PCR, western blotting and biochemical assays analysis. Transcriptomic and epitranscriptomic changes following ZC3H13 depletion were assessed by RNA-seq and MeRIP-seq analyses. RNA immunoprecipitation (RIP) assays and mRNA stability experiments (Act D assay) were performed to investigate m6A-dependent regulation of target transcripts.

RISULTATI

RESULTS:
ZC3H13 emerged as a critical dependency in leukemia and was correlated with poor clinical outcome. ZC3H13 is overexpressed in leukemia, and its depletion impaired cell growth across multiple cell lineages. Integrated RNA-seq and MeRIP-seq analyses reveal that ZC3H13 loss rewrites the m6A epitranscriptomic landscape and disrupts the leukemic transcriptional program. Mechanistically, ZC3H13 depletion reduced 3'UTR methylation on MYC, impairing the recruitment of the m6A reader IGF2BP3 and thus destabilized the MYC mRNA. In parallel, ZC3H13-dependent m6A remodeling increases 5'UTR methylation on MAPK3, which recruits a decaying m6A reader YTHDF2 and thus selectively degrade MAPK3 mRNA and suppress its protein expression and the downstream signaling.

CONCLUSIONI

CONCLUSION:
Our findings define a novel mechanism by which ZC3H13-m6A-IGF2BP3/YTHDF2 axis governs the leukemic oncogenic program through selective modulation in MYC and MAPK3 mRNA fate. These findings underscore ZC3H13 as a potent therapeutic vulnerability for leukemia treatment.

A083

CATP3AK: A THREE-TOOL INTEGRATED PIPELINE FOR ACCURATE PEAK CALLING IN ATAC- AND CHIP-SEQ DATA

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INTRODUZIONE

Analysis of ATAC- and ChIP-Seq data is often complex due to the large number of candidate peaks assessed during peak calling. Noise, inter-samples biological variation, and overlapping signals can reduce the accuracy of this step. To overcome these issues, we developed a Nextflow based pipeline integrating three complementary tools to identify the most reliable peaks for downstream analyses.

METODI

Raw ATAC- and ChIP-Seq data can be preprocessed assessing the quality with FastQC and trimmed automatically using trimgalore. Then, data are aligned against the preferred reference using bowtie2 and the resulting files are sorted, deduplicated and indexed. Peak candidates are then analyzed using three independent tools: MACS3, Lanceotron, omnipeak. While MACS3 relies on statistical modeling, LanceOtron utilizes deep learning to recognize and extract peak shapes, and omnipeak uses Hidden Markov Models (HMM). Integrated application of these tools significantly improves precision and accuracy in peak calling. Finally, the peaks are annotated, and all the results are plotted with custom plots, while MultiQC shows all the statistics and data quality plots.

RISULTATI

The pipeline, called CATP3ak, shows an improvement in peaks selection thanks to the three different tools respect to a conventional single-tool approach. ATAC and ChIP-Seq data show a reduced background noise and redundant signals while highlighting possible biologically relevant peaks. The combined strategy can detect peaks called univocally in one tool or in more than one, increasing overall confidence. Moreover, the deep learning and the HMM tools identify low-intensity peaks frequently missed by standard filtering methods. Overall, this pipeline reduces running time and generates a refined set of peaks ready for other statistical analyses.

CONCLUSIONI

We present CATP3ak, a Nextflow pipeline for ATAC- and ChIP-Seq analysis based on the integration of three complementary peak-selection tools. By combining statistical filtering, neural networks, and HMM, CATP3ak improves the accuracy and reproducibility of peak identification while reducing false positives. The modular structure of the pipeline allows adaptability to different user needs and helps with a more accurate biological interpretation.

A084

DOSKI-NF: AN INTEGRATED COMPUTATIONAL PIPELINE FOR CONSENSUS VARIANT CALLING AND COMPREHENSIVE CANCER GENOMIC PROFILING

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INTRODUZIONE

The widespread use of next generation sequencing in cancer genomics has generated numerous bioinformatics tools with variable performance, potentially affecting downstream analyses and clinical decision-making. We developed a computational pipeline for the analysis of matched germline and somatic cancer samples, aimed at (i) building and validating a robust The Cancer Genome Atlas (TCGA)-like framework and comprehensive genomic profiling, including tumor mutational burden (TMB) and microsatellite instability (MSI); and (ii) evaluating 20 different variant callers to optimize the workflow and fine-tuning optional features for specific sample types, i.e., Formalin-Fixed Paraffin-Embedded (FFPE) cancer samples.

METODI

We implemented a Nextflow workflow to process whole-exome sequencing short-reads data from FASTQ or BAM files and generate user-friendly output results. Germline and somatic variant calling are performed in parallel, and the resulting VCFs are integrated through a consensus strategy retaining only variants supported by at least two different callers. A preliminary benchmarking was performed on two breast cancer datasets, one from fresh tissue and one from FFPE tissue, by comparing results from 20 callers against ground truth sets.

RISULTATI

The TCGA-like pipeline integrating complementary profiling was successfully implemented and is currently being employed for the comprehensive molecular characterization of in-house cancer samples. Variant calling performance varied substantially across the 20 evaluated tools and depended on the dataset analyzed. The consensus-based approach across multiple variant callers consistently improved performance by balancing precision and recall. In the fresh tissue dataset, systematic evaluation among all tested combinations showed that the best combination was ClairS-TO paired with DeepSomatic. In contrast, performance in the FFPE dataset was generally lower and at least four callers are required.

CONCLUSIONI

This workflow may improve the robustness and reproducibility of variant calling while enabling comprehensive genomic characterization, advancing the clinical translation of cancer genomic results. Benchmarking is currently being expanded to additional datasets derived from different tissues and experimental methodologies, and further filtering strategies will be implemented to reduce variant calling errors, specifically in FFPE cancer samples.
Study supported by AIRC (MFAG30294) to VS.

A085

THE ONCOGENIC REPROGRAMMING OF HUMAN BASAL KERATINOCYTE: INVOLVEMENT OF FGFR2C/TRPA1 CROSSTALK WITHIN LIPID RAFT

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INTRODUZIONE

Aberrant expression of the mesenchymal isoform of FGFR2 (FGFR2c), resulting from FGFR2 isoform switching, promotes epithelial-to-mesenchymal transition (EMT) and tumorigenic features in human keratinocytes. Emerging evidence suggests an oncogenic crosstalk between FGFR2c and TRPA1, a cation channel involved in epidermal homeostasis, although its contribution to keratinocyte transformation remains unclear. This study aimed to investigate the biological and oncogenic relevance of the FGFR2c/TRPA1 axis in human basal keratinocytes.

METODI

Ultrastructural and immunofluorescence analyses were performed in both 2D and 3D culture models to evaluate the effects of TRPA1 depletion on the phenotype induced by FGFR2c overexpression. Biochemical and molecular analyses were used to assess the modulation of epithelial and mesenchymal markers associated with EMT. Lipidomic profiling was carried out to investigate membrane lipid remodeling and the regulation of inflammatory and anti-inflammatory mediators. Functional in vitro assays were performed to evaluate the acquisition of an invasive phenotype, while co-immunoprecipitation experiments assessed FGFR2c localization within lipid rafts.

RISULTATI

TRPA1 depletion counteracted the mesenchymal-like phenotype induced by FGFR2c overexpression, restoring cell-cell adhesion and reducing scattered growth in non-tumorigenic keratinocytes. TRPA1 silencing also impaired multiple FGFR2c-driven oncogenic features, including the modulation of epithelial and mesenchymal markers associated with EMT. Lipidomic analyses supported a cooperative role of FGFR2c and TRPA1 in membrane lipid remodeling and in the regulation of mediators linked to EMT and invasive behavior. Functional in vitro assays demonstrated that FGFR2c/TRPA1 crosstalk promotes the acquisition of an invasive phenotype in non-tumorigenic keratinocytes. Moreover, co-immunoprecipitation experiments showed that TRPA1 is required for FGFR2c localization within lipid rafts, highlighting the importance of membrane compartmentalization in FGFR2c-mediated oncogenic signaling.

CONCLUSIONI

These findings identify TRPA1 as a critical mediator of FGFR2c-driven keratinocyte transformation and suggest that simultaneous targeting of TRPA1 and lipid raft-associated signaling pathways may represent a promising therapeutic strategy for epithelial malignancies characterized by FGFR2 isoform switching.

A086

TLR4: A POTENTIAL THERAPEUTIC TARGET FOR THE PREVENTION OF HEMOLYTIC UREMIC SYNDROME IN CHILDREN

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INTRODUZIONE

Childhood infections by Shiga toxin (Stx)-producing Escherichia coli (STEC) cause bloody diarrhea and the life-threatening hemolytic uremic syndrome (HUS). Stx2 produced by STEC in the gut is released into the bloodstream and interacts with circulating cells (monocytes, neutrophils and platelets) through two different receptors: Gb3Cer (globotriaosylceramide) and Toll-like receptor 4 (TLR4). As a consequence, blood cells form leukocyte/platelet or platelet aggregates and release pathogenic extracellular vesicles (EV) containing Stx2 which, by damaging the kidney, induce the transition from bloody diarrhea to HUS.

METODI

Human blood was incubated with 2 nM Stx2 in the presence or in the absence of a specific monoclonal anti-TLR4 antibody (anti-TLR4, 10 µg/mL), then (i) the number of neutrophil/platelet or platelet aggregates/100 neutrophils was evaluated in blood smears stained with May-Grünwald Giemsa; and (ii) pathogenic blood cell-derived EV were isolated by exclusion size chromatography with qEV 70 nm columns or differential centrifugation, their number and size determined by nanoparticle tracking analysis, and their toxic effect on Vero cells assessed with a viability assay.

RISULTATI

In the presence of anti-TLR4, the formation of neutrophil/platelet and platelet aggregates was greatly reduced compared to the treatment with Stx2 (Stx2+anti-TLR4=10.14%±10.76 vs Stx2=100%; $p<0.0001$, n=3, and Stx2+anti-TLR4 24.84±35.13 vs Stx2=100%; $p=0.0207$, n=3, respectively). Moreover, anti-TLR4 significantly decreased the total mass of pathogenic blood cell-derived EV produced by Stx2 and, in particular, of the larger (>200 nm) EV populations (Stx2+anti-TLR4 EV=26.9%±33.8 vs Stx2 EV=100%; $p=0.0201$, n=3). Furthermore, Stx2+anti-TLR4 EV were less toxic to Vero cells compared with Stx2 EV (ID50=0.13 µL vs ID50=0.04 µL, respectively; significant y-intercept difference of the straight lines, $p=0.0042$).

CONCLUSIONI

These findings suggest that the binding of Stx2 to TLR4 is crucial for the production of cell aggregates and pathogenic Stx2-containing blood cell-derived EV. Since Stx2 associated with EV through TLR4 is responsible for the development of HUS, targeting TLR4 could be considered an interesting therapeutic option for the prevention of HUS in STEC-infected patients.

A087

TARGET THERAPY IN MULTIPLE SCLEROSIS: LONGITUDINAL ANALYSIS OF LYMPHOCYTES POPULATIONS

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INTRODUZIONE

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterized by demyelination and neurodegeneration. Although traditionally considered T-cell mediated, increasing evidence supports a central role for B cells in disease pathogenesis, not only through antibody production but also via interactions with T lymphocytes. The efficacy of anti-CD20 therapies further highlights their importance, while variability in B-cell repopulation and clinical outcomes suggests that fixed dosing regimens may not be optimal for all patients, supporting the need for personalized treatment strategies. The aim of this study was to evaluate the impact of anti-CD20 therapy on immune profiles and clinical outcomes in MS, focusing on the relationship between B-cell repopulation kinetics and the risk of progression independent of relapse activity (PIRA), as well as changes in T-cell populations.

METODI

Peripheral blood samples from MS patients (n=61) were analyzed before and after treatment with anti-CD20 therapies (ocrelizumab or ofatumumab) using flow cytometry. Based on B-cell frequency and phenotype and absolute counts measured within six months after infusion, patients were classified as responders or non-responders.

RISULTATI

After therapy, all the patients showed an enrichment of B cell compartment constituted by transitional naive B cells and plasmablasts, while showed a marked reduction in memory B cells. Non-responders patients displayed a higher baseline percentage of B lymphocytes and a higher risk of PIRA. Anti-CD20 therapy was also associated with an increase in naive and central memory CD4 T cells and a reduced percentage of effector memory T cells, follicular helper T cells, suggesting a shift toward a less inflammatory immune profile.

CONCLUSIONI

These findings suggest that anti-CD20 dosing may be personalized according to baseline CD19 B-cell counts. Immunological monitoring may help identify biomarkers predictive of treatment response and support personalized therapeutic strategies in MS.

A088

STABLE 13C AND 15N ABUNDANCE AND FRACTIONATION IN TUMOR CORE, INFILTRATING FRONT, AND HEALTHY SURROUNDING TISSUES OF GLIOBLASTOMA

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INTRODUZIONE

Glioblastoma (GB) is the most common form of brain cancer, accounting for more than 80% of all malignant brain tumors. It is associated with a poor prognosis and a median survival time of approximately 12–15 months, despite the use of multimodal treatment approaches. The persistently unfavorable outcome in GB patients is partly due to significant limitations in current diagnostic methodologies, including imaging, biopsy, and the absence of reliable genetic or molecular markers.

METODI

Twenty GB newly diagnosed patients were enrolled in this study. Tissue samples were obtained intraoperatively using 5-aminolevulinic acid (5-ALA) fluorescence-guided neurosurgery, enabling precise isolation of three distinct anatomical regions: the tumor core, the infiltrating margin, and the adjacent non-tumorous brain tissue. Tissues were frozen in dry ice and lyophilized; samples were then weighed and encapsulated in tin microcapsules. Measurement of $\delta^{13}C$ and δ^{1}#N was performed by isotope ratio mass spectrometry (IRMS). The mass spectrometer, coupled with an elemental analyzer (EA), enabled combined measurement of isotopic ratios as well as carbon and nitrogen concentrations on single samples.

RISULTATI

Isotopic methodologies are widely used, leveraging the phenomenon of fractionation, which is the discrimination of the lighter isotope relative to the heavier isotope of an element occurring during biochemical reactions. Here, we observed that GB tumour tissues, as compared to non-tumorous surrounding ones, were enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$. These phenomena reflect the GB's significant metabolic reprogramming to support rapid growth and resistance to therapy. Moreover, $\delta^{13}C$ values were correlated with MGMT methylation. Comparative analysis of $\delta^{13}C$ values between healthy tissues and the tumor core revealed a lower $\delta^{13}C$ within the tumor core as compared to non-tumorous surrounding tissue in MGMT unmethylated patients, confirming the recent multiomics observation that MGMT unmethylated GB showed an enhanced lipid metabolism, generating a lipogenic environment permissive to GB survival

CONCLUSIONI

Our findings suggest that the isotope balance of ^{13C} and ^{15}N is a useful method for evaluating metabolism, because it reflects changes in C partitioning and N excretion. By adapting Isotopic Mass Spectroscopy to cancer biology, the study introduces a conceptual shift from pathway-centric analyses to a systems-level perspective on metabolism.

A089

SPATIAL TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS REVEALS IMMUNE SUPPRESSION AND MYELOID ENRICHMENT IN H3K27M DIFFUSE MIDLINE GLIOMAS

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INTRODUZIONE

Diffuse midline gliomas (DMGs) harbouring H3K27M mutations are aggressive brain tumours characterized by profound epigenetic dysregulation and an immunologically cold tumour microenvironment (TME). These tumours sustain an OPC-like malignant program alongside AC-like and MES-like transcriptional states, while their spatial organization and immune interactions remain poorly defined.

METODI

We applied Xenium spatial transcriptomics to 11 paediatric FFPE biopsies using a brain panel supplemented with 100 custom probes, including H3K27M variants (H3.3 and H3.1). Single-cell proteomic profiling was performed with PhenoCycler-Fusion on the same sections and on 11 additional predominantly adult FFPE samples. Integrated transcriptomic–proteomic analyses were conducted across overlapping cases.

RISULTATI

H3.3K27M transcripts were detected in all samples, while H3.1K27M was absent. Unsupervised clustering identified 13 cellular populations. Malignant cells exhibited OPC-like and AC-like programs, with a minor MES-like population partitioned into hypoxic and non-hypoxic subprograms, suggesting spatially distinct tumour niches. OC-like malignant cells were not observed, and oligodendrocytes were confined to the non-malignant compartment, indicating limited differentiation along this lineage. Tumour cells expressed GABAergic neuronal signatures, supporting a role for GABAergic signalling in DMG proliferation. The TME was dominated by microglia and macrophages, with minimal lymphoid infiltration. Gene ontology analyses revealed stemness pathways in OPC-like cells, immune-suppressive programs in AC- and MES-like populations, and innate inflammatory signalling in myeloid cells. Spatial integration highlighted a hybrid microglial state characterized by activation without effective lymphoid recruitment.

CONCLUSIONI

Overall, DMGs display a myeloid-enriched, lymphoid-poor TME with spatially segregated tumour programs linked to immune suppression. These findings support myeloid reprogramming as a potential therapeutic strategy in this currently incurable disease.

A090

CIRCADIAN IMMUNE MODULATION DURING INFECTIONS DRIVEN BY TRYPTOPHAN PARTITIONING

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INTRODUZIONE

Circadian rhythms influence multiple aspects of physiology and are tightly linked to metabolism, regulating daily fluctuations in hormones, metabolites, and metabolic enzymes. During infection, these rhythms shape nutrient availability and immune responses, affecting microbial colonization, inflammation, tissue damage, and host–microbiota interactions. We previously demonstrated that the host response to pulmonary infection by the opportunistic fungus Aspergillus fumigatus oscillates over a 24-hour period, driven in part by immunomodulatory metabolites such as tryptophan derivatives. Building on these findings, the present study investigates how circadian rhythms regulate tryptophan metabolism and whether this regulation impacts immune responses in the gastrointestinal tract.

METODI

To investigate circadian control of tryptophan metabolism in the gut, we performed transcriptional and metabolic analyses across the daily cycle in the intestines of WT mice and mice carrying mutations in key tryptophan-catabolizing enzymes. Using murine models of intestinal injury and Candida albicans infection at different times of the light–dark cycle, we assessed fungal colonization, inflammation, and the underlying immune responses.

RISULTATI

Our findings confirm that tryptophan metabolism in the gastrointestinal tract is under circadian regulation. Through genetic manipulation of both circadian pathways and tryptophan metabolism, we uncovered mechanisms underlying their bidirectional interaction. Notably, this crosstalk drives time-of-day–dependent differences in host responses to fungal infections. Specifically, circadian fluctuations in tryptophan-derived metabolites, including kynurenine and serotonin, activate distinct immunomodulatory pathways at specific times of the day, thereby shaping antimicrobial immunity.

CONCLUSIONI

Overall, our findings demonstrate that circadian regulation at the intersection of metabolism and immunity drives daily changes in host–microbe interactions, highlighting the potential for developing chronotherapy-based strategies against opportunistic infections.

A091

A RAS-FAMILY MEMBER DRIVES ADAPTIVE ERK1/2 ACTIVATION FOLLOWING ERK5 INHIBITION IN BRAFV600E-MUTATED MELANOMA

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INTRODUZIONE

Malignant melanoma is an aggressive cancer driven by aberrant Mitogen Activated Protein Kinase (MAPK) signalling in certain subtypes. Although inhibitors targeting BRAF and MEK1/2 improve outcomes in BRAFV600E-mutant patients, resistance frequently arises. Accumulating evidence from preclinical models points to ERK5 activation as a compensatory mechanism occurring upon RAF-MEK1/2-ERK1/2 inhibition, thereby promoting resistance to these therapeutic strategies. Here, we identify a small Ras-family GTPase (here termed X-Ras) as a key mediator of pro-survival signals that promotes the activation of the MEK1/2-ERK1/2 cascade following ERK5 inhibition in BRAFV600E-mutated melanoma cells.

METODI

BRAFV600E-mutated A375 and Sk-Mel-5 melanoma cells were silenced for ERK5 using specific shRNAs. X-RAS was silenced using siRNAs. Small-molecule inhibitors used included ERK5 inhibitors (JWG-071, XMD8-92) and pan-RAS inhibitor (RMC-6236). 3D speroid assays were performed in melanoma cells treated with RMC-6236 in combination with the ERK5 inhibitors.

RISULTATI

Using BRAFV600E-mutated A375 and Sk-Mel-5 melanoma cells, we found that genetic and pharmacological inhibition of ERK5 triggers hyperactivation of the MEK1/2-ERK1/2 cascade, accompanied by increased X-Ras expression at mRNA and protein levels, suggesting the engagement of a compensatory loop. Accordingly, X-Ras silencing prevented ERK1/2 activation and potentiated the anti-proliferative and pro-apoptotic effects of ERK5 inhibition.
To assess the translation relevance of these findings, we tested the pan-RAS inhibitor RMC-6236. RMC-6236 treatment dose-dependently reduced melanoma cell viability, and its combination with JWG-071 ERK5 inhibitor produced stronger inhibitory effects on both cell viability and 3D tumor growth compared to single treatments.

CONCLUSIONI

Overall, our results reveal a novel X-Ras-dependent mechanism driving ERK1/2 activation following ERK5 inhibition in BRAFV600E-mutant melanoma, contributing to resistance to MAPK-targeted therapies. Combined targeting of ERK5 and X-Ras may thus represent a promising strategy to enhance apoptosis and overcoming resistance in melanoma.

A092

FROM 2D TO 3D IN VITRO MODELS OF GLAUCOMA: INSIGHTS INTO MOLECULAR MECHANISMS INVOLVED IN MüLLER CELLS RESPONSE

M. Di Vito Nolfi [1], D. Vecchiotti [1], C. Compagnoni [1], I. Flati [2], F. Dall'Aglio [1], F. Veglianti [1], D. Verzella [1], D. Capece [1], A. Tessitore [1], E. Alesse [1], F. Zazzeroni [1]

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INTRODUZIONE

Glaucoma represents the second cause of blindness in the world. Understanding the molecular mechanisms that lead to cellular degeneration and vision loss is crucial to develop novel and effective therapeutical strategies. Despite it is known that the triggering event in glaucoma is an elevated intraocular pressure (IOP), subsequent inflammation and oxidative stress strongly contribute to neuronal injury and retinal degeneration. In this context, Müller cells in neuroretinal layer play a relevant role, supporting retinal ganglion cells but also contributing to pathophysiology of glaucoma. Elucidating molecular mechanisms involved in the response to IOP is therefore critical to preserve their functionality and countering neuronal degeneration. In order to elucidate the molecular mechanisms involved in the response to IOP, we generated an advanced model of disease using 3D bioprinting technology in addition to conventional 2D cell cultures. We also evaluated a neurotrophic compound (NTRC) as potential therapeutical agent.

METODI

The co-culture system constituted by bioink-embedded Rat Retinal Müller Cells (rMC-1) on top of ARPE19 cell monolayer was designed to mimic neuroretinal and retinal pigment epithelium layers respectively. rMC-1 bioprinting was performed by using CELLINK BIO X 3D bioprinter and glaucomatous condition was reproduced with millifluidic LiveFlow bioreactor (IVTech). Proteomic and viability analysis were performed by Western blot and PrestoBlue assay respectively.

RISULTATI

Integrating 2D and 3D models we identified Akt/mTOR, STAT3, and NF-κB pathways as molecular mechanisms involved in response to the increase of hydrostatic pressure and highlighted the crucial role of mTOR signaling in oxidative stress response. Interestingly, 2D and 3D models showed both similar and different responses to hydrostatic pressure. Moreover, we also observed that NTRC modulates these signaling and appears to reduce oxidative stress in a context-dependent manner.

CONCLUSIONI

Using 2D and 3D models of Müller cells we identified several pathways involved in the response to increased hydrostatic pressure and highlighted a potential role of NTRC in reducing oxidative stress. Importantly, different responses between 2D and 3D cultures underline the importance of using 3D and co-culture systems.

A093

THE ERα/EGFR/FILAMIN A SIGNALING NEXUS: A NEW DRIVER OF CASTRATION-RESISTANT PROSTATE CANCER

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INTRODUZIONE

Prostate cancer (PC) progression toward castration-resistant prostate cancer (CRPC) is sustained by the activation of interconnected steroid hormone- and growth factor-dependent signaling pathways that promote tumor aggressiveness, metastatic dissemination, and therapeutic resistance. In particular, extranuclear estrogen receptor (ER) signaling has emerged as a critical regulator of rapid non-genomic pathways, frequently converging with EGFR-mediated oncogenic signaling. Filamin A (FlnA), a scaffold protein involved in cytoskeletal remodeling and signal transduction, has also been implicated in cancer cell motility and metastatic behavior. However, the existence and functional relevance of a dynamic ERα/EGFR/FlnA signaling complex in PC remain poorly understood. This study investigated the role of the ERα/EGFR/FlnA complex in prostate cancer using 2D and 3D models.

METODI

Normal prostate epithelial cells and PC cells (LNCaP, DU-145, and PC-3) were cultured (1) and stimulated with 17β-estradiol (E2, 10 nM), EGF (100 ng/mL), and/or the EGFR inhibitor AG1478. Protein interactions and localization were evaluated by co-immunoprecipitation and immunofluorescence assays (2). Functional assays assessing cell viability, migration, and spheroid growth were also performed.

RISULTATI

Co-immunoprecipitation analyses revealed a rapid association among ERα, EGFR, and FlnA following short-term stimulation with either E2 or EGF (5 min), particularly in DU-145 cells. Immunofluorescence analyses confirmed the extranuclear co-localization of these proteins upon stimulation, supporting the activation of rapid non-genomic signaling pathways. Functional assays demonstrated that both E2 and EGF promote prostate cancer cell viability, migration, and spheroid growth. Preliminary observations suggest that the ERα/EGFR/FlnA signaling axis may contribute to these aggressive phenotypes.

CONCLUSIONI

Our findings support the existence of a rapid extranuclear ERα/EGFR/FlnA signaling complex, potentially involved in prostate cancer progression and aggressive behavior, highlighting a potential therapeutic target in advanced disease.

A094

INVESTIGATING THE ROLE OF CARBOXYLESTERASE 1 (CES1) IN SPP1+-TUMOR-ASSOCIATED MACROPHAGES (TAMS)

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INTRODUZIONE

Carboxylesterase 1 has been identified as an NF-κB-regulated lipase that regulates metabolic adaptation and survival in colorectal cancer (CRC). Data generated in our laboratory underline a similar CES1-dependent mechanism in High-Grade Serous Ovarian Cancer (HGSOC). However, we could not exclude additional roles of CES1 in the symbiosis between tumour microenvironment (TME) and cancer cells that fuel tumour progression and metastasis. The analysis of scRNA data from patients with CRC and EOC showed a high CES1 expression in specific subsets of tumour-associated macrophages (TAMs), including $SPP1^+$-TAMs, which are associated with poor prognosis. These findings suggest a potential role for CES1 in creating an immunosuppressive niche that favours tumour aggressiveness and therapy resistance.

To investigate the role of CES1 in $SPP1^+$-TAMs and assess whether its inhibition could reprogram them toward an anti-tumour phenotype and ultimately reduce tumour growth.

METODI

First, we established a 3D co-culture model using HGSOC cells and macrophages differentiated protocol to generate $SPP1^+$-TAMs. We monitored spheroid growth and cell viability and characterized the molecular profiles of wild-type and CES1 knockout macrophages, as well as HGSOC cells.

RISULTATI

We found that $SPP1^+$-TAMs are able to strongly promote the proliferation rate of HGSOC cells into the spheroid, while this effect is impaired when HGSOC cells are cultured with CES1-depleted $SPP1^+$-TAMs. In addition, molecular analysis performed after cell type separation revealed that CES1 silencing is associated the downregulation of MARCO, a well-established of $SPP1^+$-TAMs and the increase of PDL1. Interestingly, the molecular reprogramming of CES1-depleted TAMs also affects HGSOC cells behaviour.

CONCLUSIONI

These data sustain the emerging relevance of CES1 in aggressive subset of TAMs, suggesting that targeting this lipase could afford a dual benefit in the management of HGSOC.

A095

PERIPHERAL IMMUNE SIGNATURES ASSOCIATED WITH PROGRESSION INDEPENDENT OF RELAPSE ACTIVITY IN MULTIPLE SCLEROSIS

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INTRODUZIONE

Progression Independent of Relapse Activity (PIRA) is the main driver of irreversible disability accumulation in multiple sclerosis (MS), reflecting chronic neurodegenerative processes and compartmentalized central nervous system inflammation independent of acute disease activity. Since early PIRA is associated with poor prognosis and is currently identifiable only retrospectively through EDSS worsening, predictive biomarkers remain a major unmet need.

METODI

This study aimed to identify cellular immune profiles as potential early prognostic biomarkers of PIRA in patients with multiple sclerosis. Peripheral blood samples from MS patients with at least 15 years of disease duration were analyzed by flow cytometry. Patients were classified as PIRA (disability accumulation attributable to PIRA and 4–5 relapses during the first 3 years of disease) or NO_PIRA (no disability accumulation and 1–2 relapses during the first 3 years).

RISULTATI

PIRA patients showed reduced memory B cells and increased naïve and transitional B cells, suggesting a shift toward immature phenotypes and a chronically altered immune response, potentially compartmentalized within the central nervous system. Increased CD4+ Th2 and central memory subsets, together with expansion of CD8+ CD95+ T cells, with an increase of memory cells compartment and reduced CD8+ naïve T cells, indicating an immune cell activation and memory maintenance. The $\gamma\delta$ T-cell compartment displayed increased TIM3 expression in both Vδ1 and Vδ2 T cells and a reduced frequency of effector Vδ2+ T cells subset, consistent with chronic activation and impaired cytotoxic responses. Moreover, expansion of NK CD57+ cells supported a profile characterized by chronic activation, terminal differentiation, and immune senescence.

CONCLUSIONI

PIRA patients display a peripheral immune profile characterized by chronic activation, immunosenescence, and exhaustion involving multiple immune compartments. These findings support distinct immunopathological mechanisms underlying PIRA and identify immune signatures that may serve as prognostic biomarkers and therapeutic targets.

A096

BELINOSTAT ENHANCES CRIZOTINIB EFFICACY IN ADVANCED ALK# ALCL SPHEROID AND CAM MODELS

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INTRODUZIONE

Anaplastic large cell lymphoma (ALCL) is an aggressive subtype of peripheral T-cell lymphoma frequently characterized by oncogenic ALK activation. Although the ALK inhibitor crizotinib shows remarkable clinical efficacy in ALK+ ALCL, the emergence of drug resistance remains a major limitation. Increasing evidence suggests that tumor microenvironment-mediated protection and adaptive survival mechanisms contribute to therapeutic failure. Since histone deacetylase inhibitors (HDACi) can modulate transcriptional and survival programs, we investigated whether the HDAC inhibitor belinostat could enhance the anti-tumor activity of crizotinib in advanced ALK+ ALCL models.

METODI

ALK+ ALCL cell lines were cultured as 3D spheroids, including tumor-only spheroids and multicellular spheroids generated in the presence of stromal components. Spheroids were treated with crizotinib and belinostat, alone or in combination, and spheroid growth, morphology, and viability were evaluated. The anti-tumor activity of combined treatment was further investigated in vivo using the chorioallantoic membrane (CAM) assay. Tumor growth and dissemination to the bone marrow were assessed by histological and molecular analyses.

RISULTATI

In tumor-only spheroids, crizotinib reduced growth and viability, whereas belinostat alone exerted limited activity. Notably, combined treatment induced a marked and significant reduction in spheroid size and viability compared to single agents. In multicellular spheroids, stromal components reduced crizotinib efficacy, confirming a protective microenvironmental effect. Importantly, the combination of crizotinib and belinostat overcame stromal-mediated resistance and restored drug sensitivity. In CAM assays, combined ALK and HDAC inhibition significantly reduced tumor growth and dissemination to the chicken-embryo bone marrow compared to monotherapies.

CONCLUSIONI

Combined ALK and HDAC inhibition exerts synergistic anti-tumor activity in ALK+ ALCL and effectively overcomes microenvironment-mediated resistance in 3D and in vivo models. These findings support the therapeutic potential of combining crizotinib and belinostat as a novel strategy for ALK+ ALCL treatment.

A097

THE MEDITERRANEAN DIET AS A HEALTHY AGING STRATEGY: RESULTS FROM THE EUROPEAN NU-AGE PROJECT ON BODY COMPOSITION

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INTRODUZIONE

Population ageing in Europe is rapidly accelerating, with individuals over 65 expected to reach 28.1% by 2050. This shift is driving a major increase in age-related disease burden and healthcare costs. Identifying effective nutritional strategies to promote healthy ageing is therefore urgent, particularly in pre-frail older adults, a highly prevalent and vulnerable group.

METODI

The NU-AGE study is a large-scale, multicentre, randomized controlled trial conducted across five European countries. A total of 1,246 community-dwelling adults aged 65–79 years were enrolled and followed for one year under a modified Mediterranean dietary intervention. Body composition was assessed using DXA at baseline and after 12 months. Analyses included 1,139 participants with complete datasets.

RISULTATI

While no overall changes in body weight or BMI were detected, the intervention induced clinically relevant improvements in body composition. Specifically, significant reductions in total and abdominal fat-to-lean mass ratios were observed, with the strongest effects in the Italian cohort. In parallel, skeletal muscle index improved significantly in this subgroup. Importantly, these changes were associated with an improvement in frailty status.

CONCLUSIONI

Although body weight remained unchanged, the NU-AGE dietary intervention significantly improved body composition and frailty outcomes in older adults. The magnitude of response varied across countries, highlighting inter-population differences in dietary responsiveness. These results support the concept that nutritional interventions can modulate metabolic processes underlying age-related tissue remodeling, including fat redistribution and muscle preservation. DXA-derived metrics may therefore serve as valuable biomarkers to investigate the pathophysiology of frailty and the metabolic mechanisms linking nutrition to healthy ageing.

A098

BRCA-ASSOCIATED AND HRD-LIKE SIGNALS DEFINE DISTINCT TUMOR MICROENVIRONMENT STATES IN MALE BREAST CANCER

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INTRODUZIONE

Male breast cancer (MBC) is rare and heterogeneous, with limited biomarkers for risk stratification and treatment decisions. Transcriptome-based tumor microenvironment (TME) classification may capture immune and stromal states that shape the impact of genomic instability. We applied the Molecular Functional Portrait (MFP) RNA-seq framework to stratify MBC tumors and assess BRCA status, homologous recombination deficiency (HRD)-related signatures and cGAS-STING activation across TME subtypes.

METODI

RNA-seq data from 98 MBC tumors were classified using the MFP pipeline. Immune and stromal composition was inferred by MCP-counter and CIBERSORTx. Germline BRCA pathogenic variants (n=29) were mapped to MFP classes. Genomic instability was assessed using published transcriptomic HRD signatures, and innate sensing using a cGAS-STING activation signature. Group differences were tested using Fisher's exact, Kruskal-Wallis and Wilcoxon rank-sum tests.

RISULTATI

Four MFP classes were identified: immune-enriched (IE, n=23), immune-enriched/fibrotic (IE/F, n=11), fibrotic (F, n=19) and immune-depleted (D, n=45). BRCA-mutated tumors were enriched in IE (10/23) and D (17/45), and nearly absent from fibrotic states (Fisher p=0.007). HRD-related scores were higher in non-fibrotic classes (IE and D) than in fibrotic-associated classes (IE/F and F; p=8.50×10^-6). This enrichment persisted among BRCA wild-type tumors (p=2.91×10^-4), suggesting a BRCAness-like phenotype involving both BRCA-mutated and HRD-high BRCA wild-type cases. The cGAS-STING signature was strongly stratified across MFP states (p=8.33×10^-7) and separated IE from D tumors (p=6.18×10^-9). Deconvolution supported the MFP classification, with higher CD8+ T-cell signals in IE and increased fibroblast/endothelial components in fibrotic states.

CONCLUSIONI

In MBC, BRCA-mutated tumors and HRD-like transcriptional signals are embedded within distinct TME subtypes. Genomic instability may therefore translate into divergent immune states, with cGAS-STING-associated activation contributing to the separation between immune-inflamed and immune-depleted tumors. Integrating BRCA status, HRD-like signals and functional TME classification may improve biological stratification and support biomarker discovery.

A099

RICIN-INDUCED INTESTINAL TOXICITY: EVALUATION OF PATHOGENETIC MECHANISMS ON BARRIER INTEGRITY, REDOX BALANCE, AND CYTOKINE EXPRESSIONS IN HUMAN INTESTINAL CELL LINES

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INTRODUZIONE

Ricin is a highly potent type 2 ribosome-inactivating protein, scheduled by Chemical Weapons Convention as chemical weapon in Schedule 1A and by Biological and Toxins Weapons Convention as Category B substance. Ricin cytotoxicity is driven by its lectin B-chain, which enables cell entry, and its enzymatic A-chain, which can depurinate different polynucleotide substrates, triggering multiple cell death pathways. Although the systemic toxicity of ricin has been extensively studied, its localized effects on the gastrointestinal tract remain a critical concern, particularly following oral exposure. The aim of this study is to further elucidate ricin toxicity mechanisms in human intestinal cell lines.

METODI

To accurately mimic the physiological human intestinal barrier, complex co-culture models of Caco-2 and HT29 cells were established on Transwell inserts. Barrier integrity was monitored through Transepithelial Electrical Resistance (TEER) measurements. Additionally, the involvement of oxidative stress in ricin-treated cells was directly quantified through cytofluorimetric analysis of reactive oxygen species (ROS) in Caco-2 and HT29 monolayers and indirectly investigated by pretreating cells with ROS scavengers. The inflammatory response was evaluated by measuring the levels of the pro-inflammatory cytokines IL-6 and IL-8.

RISULTATI

Biological assays showed that ricin significantly reduced TEER values, suggesting a compromised barrier integrity even in complex co-culture models. Treatment with ricin induced a marked cellular redox imbalance; notably, ricin cytotoxicity could be completely prevented by pre-incubating cells with ROS scavengers. Moreover, ricin exposure stimulated a significant increase in the pro-inflammatory IL-6 and IL-8 levels.

CONCLUSIONI

These findings may establish a possible link between the intestinal barrier disruption, oxidative damage and the development of a pro-inflammatory microenvironment in intestinal cell lines. Overall, these results clarify the mechanisms underlying ricin-induced intestinal pathogenesis and pave the way for new pharmacological strategies to counteract oral ricin intoxication.

A100

BIOLOGICAL VERSUS CHRONOLOGICAL AGE IN SICILIAN CENTENARIANS: EVALUATION THROUGH PHENOTYPIC AGE

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INTRODUZIONE

Exceptional longevity is frequently associated with heterogeneity in biological aging trajectories [1]. Chronological age alone may not adequately reflect the functional and physiological status of centenarians. Phenotypic Age (PhenoAge), developed by Levine et al. [2], integrates chronological age with clinical biomarkers associated with mortality risk and aging-related decline, providing an accurate estimate of biological aging.

METODI

A cohort of 41 Sicilian centenarians, comprising semi- and supercentenarians aged between 100 and 111 years, was evaluated. For each subject, PhenoAge was calculated according to [2]. The analysis is based on chronological age and nine clinical biomarkers, including albumin, creatinine, glucose, C-reactive protein, lymphocyte percentage, mean corpuscular volume, red cell distribution width, alkaline phosphatase, and white blood cell count. Subjects were subsequently stratified according to the discrepancy between chronological age and biological age into biologically younger and biologically older groups.

RISULTATI

The cohort showed a mean chronological age of 104.1 ± 3.4 years, while the mean PhenoAge was 101.8 ± 7.8 years. Despite the extreme chronological age, substantial inter-individual variability in biological aging emerged. 27 subjects were classified as biologically younger, whereas fourteen exhibited accelerated phenotypic aging. The difference between chronological age and PhenoAge ranged from −17.7 to +14.8 years. Correlation analysis demonstrated a significant but moderate association between chronological and biological age ($r=0.32$; $p=0.043$), suggesting that chronological age only partially reflects biological aging processes in extreme longevity. Biologically younger individuals showed markedly lower PhenoAge values compared with biologically older counterparts.

CONCLUSIONI

These findings support the concept that centenarians represent a biologically heterogeneous population. Some individuals appear phenotypically younger despite advanced chronological age, suggesting the presence of protective mechanisms associated with healthy aging and resilience. PhenoAge may represent a useful tool for characterizing biological aging trajectories in long-lived populations and identifying biomarkers associated with successful aging.

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A101

PLASMA- AND TISSUE-BASED MIRNOME PROFILING OF MALE BREAST CANCER CASES FOR THE IDENTIFICATION OF NOVEL MOLECULAR BIOMARKERS

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INTRODUZIONE

Male breast cancer (MBC) is a rare disease with still undefined clinical management. Pathogenic variants (PVs) in BRCA1/2 are established risk factors and may guide personalized treatment. Liquid biopsy is a minimally invasive approach for identifying molecular biomarkers, and circulating microRNAs (cmiRNAs) are particularly promising. This study aimed to perform a comprehensive miRNome analysis in plasma and tissue samples to identify biomarkers associated with MBC risk and to compare miRNA profiles between MBC patients with and without BRCA PVs, improving early detection and clinical management.

METODI

Plasma samples from 129 MBCs (30 BRCA+, 99 BRCA−) and 100 healthy male controls (all BRCA−) were analyzed. Clinicopathological data were available for all MBCs. FFPE samples from 58/129 MBCs (8 BRCA+, 50 BRCA−) were also included. MiRNAs were isolated using commercial kits, and RNA quality and quantity were assessed with Qubit Flex 4.0 and 2100 Bioanalyzer. MiRNome profiling was performed using the QIAseq miRNA Library Kit and libraries were sequenced on the Illumina MiSeq i100+ platform. Sequencing runs passed quality control for reliability. Differential expression analysis was performed using RNA-seq Analysis portal, followed by downstream analyses in R.

RISULTATI

In case-control analysis, 296 cmiRNAs were significantly differentially expressed (DE) in plasma samples from MBCs compared with controls. A logistic regression model identified an 8-miRNA signature (AUC=0.971) able to discriminate the two groups; six miRNAs were upregulated and two downregulated in MBCs. The model was validated in an external female BC cohort (AUC=0.875).
In case only analysis, 39 cmiRNAs were DE in plasma samples from BRCA+ compared with BRCA− MBCs. Preliminary analysis of 58 FFPE samples identified 27 DE miRNAs between the two groups. Hsa-miR-187-3p and hsa-miR-4508 were DE in both plasma and tissue analyses. All identified DE miRNAs are involved in tumorigenesis-related pathways.

CONCLUSIONI

These findings suggest that cmiRNAs may represent potential biomarkers for MBC. The 8-miRNA signature may support MBC risk evaluation. Moreover, miR-4508 and miR-187-3p could distinguish MBCs with and without BRCA PVs, supporting early diagnosis and clinical management, although validation in larger cohorts is required.

A102

BEYOND NK CELL EXPANSION: CMV AND INFLAMMAGEING SHAPE INNATE IMMUNE REMODELLING IN EXTREME LONGEVITY

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INTRODUZIONE

Immunosenescence and inflammageing are strongly influenced by chronic antigenic stimulation, particularly cytomegalovirus (CMV) infection [1,2]. Previous studies in Sicilian semi- and supercentenarians reported an age-related increase in circulating Natural Killer (NK) cells, suggesting a possible adaptive role of innate immunity in longevity [3]. The present study aimed to investigate further whether age-related changes in NK cells, monocytes, and neutrophils are directly associated with chronological ageing or rather influenced by sex, systemic inflammation, and CMV serostatus.

METODI

A total of 54 Sicilian individuals aged 19–110 years were enrolled and stratified by age group. Monocyte and neutrophil counts were determined using automated haematological analysis, while NK cells (CD3−CD16+CD56+) were quantified by flow cytometry. Serum IL-6 levels and anti-CMV IgG titres were measured using chemiluminescent immunoassays. Multiple negative binomial regression models were employed to assess the independent contributions of age, sex, CMV serostatus, and systemic inflammation (IL-6) to variations in NK-cell, monocyte, and neutrophil counts.

RISULTATI

After adjustment for covariates, NK cell counts showed only a non-significant trend toward increase with age, whereas sex was significantly associated with higher NK cell levels (p=0.025). Monocyte counts were positively associated with IL-6 levels (p=0.016), while no independent effect of age emerged. Neutrophil counts were not associated with age but were significantly reduced in subjects with high anti-CMV titres (p=0.020). No significant association between NK cells and CMV or IL-6 was detected.

CONCLUSIONI

These findings suggest that innate immune remodelling in extreme longevity is not solely driven by chronological ageing. Consistent with previous reports of NK-cell expansion in centenarians [3], these data identify sex-related immune dimorphism and inflammageing as key factors shaping innate immune profiles in long-lived individuals [3,4]. Increased NK-cell and monocyte counts appear linked to sex-related immune differences and chronic low-grade inflammation, reflected by elevated IL-6 levels, rather than ageing itself. Overall, the innate immune landscape in extreme longevity likely results from the interplay between chronic antigenic stimulation, inflammatory burden, and host-specific factors.

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A103

DOSE-DEPENDENT RESPONSE OF HUMAN PBMC VIABILITY BY OLEUROPEIN TREATMENT: PRELIMINARY DATA FOR SENOMODULATORY APPLICATIONS

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INTRODUZIONE

Cellular senescence and immunosenescence are key drivers of inflammaging and age-associated functional decline [1]. Increasing evidence links oxidative stress–driven senescence pathways to these processes. Natural polyphenols, including oleuropein (OLE), have gained attention for their antioxidant and cytoprotective properties [2,3]. This first step of the study aimed to identify the suitable OLE concentration range for the optimal viability of PBMCs, for the subsequent evaluation of the senomodulatory effect of OLE on PBMC oxidative stress-induced senescence.

METODI

PBMCs were isolated from buffy coats by density-gradient centrifugation and cultured under standard conditions. Cells were exposed to increasing concentrations of OLE (1, 5, 15, 25, 50, 75 and 100 μM) for 24 and 48 h. Cell viability was assessed using Trypan Blue exclusion and XTT assays. Data were normalized to untreated controls.

RISULTATI

OLE induced dose- and time-dependent effects on PBMC viability. At 24 h, low and intermediate concentrations (1–15 μM) maintained cell viability comparable to controls, with 15 μM showing the most favorable viability profile. In contrast, higher concentrations (≥50 μM) induced a progressive reduction in viable cells. After 48 h exposure, cytotoxic effects became more pronounced, particularly at concentrations 75–100 μM, where a marked decrease in cell viability was observed.

CONCLUSIONI

These preliminary findings identify 1–15 μM as a potentially non-cytotoxic concentration range of OLE in human PBMCs. The results provide the basis for future investigations into the possible senomodulatory effects of OLE in oxidative stress-induced cellular senescence and inflammaging-related experimental models.

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A104

INTERSTITIAL FLUID OF PERITUMORAL ADIPOSE TISSUE AS A NOVEL SOURCE OF PROGNOSTIC BIOMARKERS IN BREAST CANCER

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INTRODUZIONE

Preclinical and clinical studies on breast cancer (BC) have demonstrated the significant prognostic value of soluble factors produced by the tumor microenvironment (TME). In BC, the TME is mainly composed of adipose tissue (AT), which regulates BC phenotypes through soluble factors released into the extracellular compartment and capable of reaching BC cells. However, the interstitial fluid (IF) of AT has never been thoroughly investigated, and its collection still represents a major technological challenge.

METODI

Proximal (PT, located at the tumor margin) and distal (DT, 5 cm from the tumor) AT biopsies were obtained from 31 patients affected by ER+ BC. IF samples were collected from AT and analyzed for the concentration of a panel of 27 molecules using a multiplex immunoassay. uPA and progesterone receptor (PgR) levels were investigated by Western blot analysis. A microneedle array (MN) was fabricated using two-photon polymerization. Data were analyzed using R.

RISULTATI

PT-IF displayed significantly higher levels of clusters of cytokines, chemokines, and growth factors compared with DT-IF. Among these, FAP, uPA, BAFF, APRIL, IGF-1, IL-6, IL-6RA, CCL2, IFNγ, CXCL10, and IL-10 were more than 1.5-fold upregulated in PT-IF compared with DT-IF. Multivariable analyses showed that higher uPA levels in PT-IF were associated with reduced PgR expression in patients' tumor tissues. In vitro experiments demonstrated that uPA treatment reduced PgR levels in BC cells. Finally, a novel MN-based patch was developed and extensively characterized. When applied to AT biopsies in a sandwich configuration, the device efficiently extracted IF. Molecular profiling of IF collected using the MN-based system confirmed that PT-IF contained higher concentrations of several molecules, including uPA, compared with DT-IF.

CONCLUSIONI

PT-AT holds an IF enriched in soluble mediators that may regulate the crosstalk between BC and TME. In particular, PT-IF-derived uPA appears to be involved in the regulation of PgR expression in BC, suggesting a novel role for uPA as a biomarker of BC aggressiveness. Moreover, the newly developed MN-based patch proved capable of collecting IF from AT, highlighting its potential as an innovative tool for IF extraction and analysis.

A105

DMLPA-BASED COPY NUMBER VARIATION ANALYSIS IN MALE BREAST CANCER: RESULTS FROM THE ITALIAN MULTICENTER STUDY

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INTRODUZIONE

Male breast cancer (MBC) is a rare malignancy, accounting for about 1% of all breast cancers (BC). Pathogenic single nucleotide variants (SNVs) in BRCA2 and BRCA1, explain nearly 15% of MBC cases. Our previous data in Italian population revealed pathogenic SNVs in additional genes, including PALB2 and ATM, in nearly 5% of MBCs. However, a substantial proportion of MBC, including high-risk MBCs, remain unexplained, suggesting the involvement of other genetic factors. In this context, copy number variants (CNVs), increasingly recognized as cancer-predisposing alterations, remain poorly explored in MBC.
This study aims to address this gap by analyzing CNVs in a large and well-characterized cohort of Italian MBCs.

METODI

Within the framework of the first Italian multicenter study on MBC, 413 high-risk MBCs, negative for previously tested SNVs in 50 cancer-related genes and suggestive of hereditary predisposition, including early onset disease, family and/or personal history of cancer, were selected.
This cohort underwent CNV analysis using digital MLPA, a novel high-throughput methodology, employing the NXtec D001 Hereditary Cancer Panel including 28 hereditary cancer-related genes. Sequencing was performed on Illumina Miseq i100+ platform.

RISULTATI

CNVs were identified in 14 genes out of 28, in 3.6% of MBCs (15/413 cases), including 12 deletions and three amplifications. Specifically, deletions in PALB2 (exons 11 and 2–4) were observed in 13.3% of MBCs (2/15 cases); further deletions in: APC (exon 8), ATM (exon 16), BAP1 (exon 11), BMPR1A (exon 12), BRCA2 (exon 15), CDH1 (exons 7–9), CHEK2 (exon 8), MLH1 (exon 9), MUTYH (exon 5), and PTEN (exon 1), were observed in 6.7% of MBCs (1/15 cases), each. Amplifications in CDKN2A (exon 2), MSH2 (exon 8), and TP53 (exon 7) were observed in 6.7% of MBCs (1/15 cases), each.

CONCLUSIONI

Our preliminary findings showed that CNVs may contribute to genetic susceptibility to MBC. Notably, PALB2 emerged as the most frequently altered gene, underscoring the importance of extending routine CNV screening beyond BRCA1/2 in MBCs with potential implications for genetic counseling, risk assessment, and precision oncology. Ongoing analyses will further define the contribution of CNVs extending the analysis to additional cases.
Study supported by AIRC (IG28775) to L.O

A106

A RAPID, SENSITIVE, AND ACCESSIBLE METHOD FOR RICIN DETECTION IN RICINUS COMMUNIS OIL EXTRACTION BYPRODUCTS TO PREVENT LETHAL INTOXICATION

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INTRODUZIONE

Ricinus communis L. represents a highly valuable agricultural and economic resource worldwide, but its industrial processing poses significant biosafety challenges. Thousands of tons of Ricinus oil are processed each year, leaving behind a substantial byproduct known as castor cake. This biomass is widely used as fertilizers and pesticides even in household environments. However, these applications are limited by the presence of ricin, a potent toxin that is a serious public health hazard and a risk for acute clinical pathology, characterized by fatal systemic toxicity and tissue damage, following accidental oral ingestion or the inhalation of toxic powder. Workers involved in handling of these extraction wastes are exposed to severe occupational hazards and the general public is highly vulnerable to accidental poisoning and localized environmental contamination. Here, we propose a rapid, sensitive, simple, and highly reproducible method to detect active ricin in castor cakes, providing an efficient surveillance strategy to safeguard public health and prevent lethal intoxication.

METODI

Complex matrices are processed, loaded onto acid-treated Sepharose-CL6B resin and the ricin content is spectrophotometrically evaluated after elution with galactose. This procedure is based on the ability of ricin to bind galactose exposed on the resin; thus, the elution of a peak indicates the presence of undegraded and potentially active ricin.

RISULTATI

This detection method has proven to be rapid, requiring only a few hours per sample, and highly sensitive, with a detection limit in the μg range. Furthermore, it is easily executable in any laboratory, as it does not require particularly complex and expensive equipment, ensuring reproducible quality control of byproducts derived from Ricinus communis oil extraction even in low-resource settings.

CONCLUSIONI

This method allows companies to self-monitor the preliminary quality control of all Ricinus communis-derived intermediate and final products intended for use as fertilizers or for other purposes, thereby ensuring that all products are completely free of active ricin before reaching the market. By reducing costs, processing time, and reagent consumption, this approach is highly accessible even to low-income countries, representing an effective opportunity to safeguard public health and monitor biosafety.

A107

EXTRACELLULAR SIGNAL-REGULATED KINASE 5 (ERK5) INHIBITION SENSITIZES TO EGFR-TARGETED THERAPIES BY MODULATING ITS EXPRESSION AND NUCLEAR LOCALIZATION IN HEPATOCELLULAR CARCINOMA CELLS

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INTRODUZIONE

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, and its incidence is expected to rise. Although systemic therapies are used in advanced disease, their clinical benefit remains limited, highlighting the need for additional therapeutic targets. Previous work from our laboratory showed that extracellular signal-regulated kinase 5 (ERK5) plays an important role in hepatocarcinogenesis and tumor growth, suggesting that ERK5 inhibition may represent a promising therapeutic strategy.

METODI

In this study, we investigated ERK5-dependent signaling in HCC using Huh7 and HepG2 cell lines. ERK5 expression was silenced by shRNA, and pharmacological inhibition was performed using the ERK5 inhibitor JWG-071. Cell viability was assessed by MTT and trypan blue exclusion assays. mRNA expression was analysed by RT-PCR and protein expression was evaluated by Western blotting and immunofluorescence.

RISULTATI

ERK5 knockdown increased EGFR mRNA and protein expression, accompanied by activation of downstream signaling pathways. Consistently, EGFR protein levels were elevated in cells treated with JWG-071. Combined treatment with JWG-071 and the EGFR inhibitor gefitinib reduced cell viability and 3D spheroid growth more effectively than single treatments. Since nuclear translocation of EGFR contributes to tumor progression through α/β-importin-dependent transport, we examined EGFR localization. ERK5-depleted cells showed increased nuclear EGFR levels, and combined treatment with JWG-071 and the α/β-importin inhibitor ivermectin showed higher antiproliferative effect than single treatments.

CONCLUSIONI

Overall, ERK5 inhibition increases sensitivity to EGFR targeting, supporting combined therapeutic strategies for HCC.

A108

THE MULTI-KINASE INHIBITOR JNJ-7706621 EMERGES AS A NOVEL ERK5 INHIBITOR

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INTRODUZIONE

The Mitogen-Activated Protein Kinase (MAPK) Extracellular signal-Regulated Kinase 5 (ERK5) is involved in several hallmarks of cancer and is a promising therapeutic target in several malignancies. ERK5 activation is also a known resistance mechanism to RAS/RAF/MEK1/2-ERK1/2 targeted therapies. However, currently available ERK5 inhibitors have not reached clinical relevance mainly due to their limited efficacy and the fact that they may paradoxically induce ERK5 nuclear translocation and transcriptional transactivator activity. Therefore, we aimed to identify novel putative allosteric inhibitors of ERK5 using a structure-based virtual screening approach.

METODI

Using MolPort and National Cancer Institute databases we performed an in silico screening based on the putative allosteric pocket of ERK5. In vitro kinase assays allowed to test the capability of candidate compounds to inhibit ERK5 kinase activity. Effects of the lead compound were evaluated in ovarian cancer, melanoma and hepatocellular carcinoma cell lines through cell counting, colony formation assays and 3D spheroid growth assay. ERK5 knockout (KO) clones were used to assess the specificity of the compound. Cellular fractionation and immunofluorescence analysis were used to analyse ERK5 subcellular localization.

RISULTATI

The multi-kinase inhibitor JNJ-7706621 was identified as a novel ERK5 inhibitor. In vitro kinase assays showed that JNJ-7706621 inhibits ERK5 kinase activity at sub-micromolar concentrations and higher efficacy than the commercially available ERK5 kinase activity inhibitor JWG-071 at 300 nM dose. Cell viability, colony formation ability and 3D spheroid growth were reduced in all cancer cell lines tested upon JNJ-7706621 administration. Importantly, ERK5 KO cells resulted less sensible to JNJ-7706621, supporting an on-target effect. Moreover, JNJ-7706621 did not induce ERK5 nuclear translocation.

CONCLUSIONI

We identified JNJ-7706621 as a new ERK5 kinase inhibitor that works at sub-micromolar concentrations, effectively inhibiting both ERK5 kinase activity and cell proliferation in an ERK5-dependent manner, without inducing ERK5 paradoxical activation. These results pave the way to a new class of small molecules targeting ERK5, which could achieve clinical relevance following additional preclinical evaluation.

A109

THYROID CANCER CELL-DERIVED ECTOSOMES INDUCE MACROPHAGE DIFFERENTIATION AND REMODEL THE TUMOR MICROENVIRONMENT.

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INTRODUZIONE

Papillary thyroid carcinoma generally features an indolent course, but a subset undergoes aggressive dedifferentiation into anaplastic variants. This malignant progression is heavily influenced by dynamic reciprocal crosstalk within the tumor microenvironment mediated by tumor-derived extracellular vesicles (EVs). Here, we investigated the cargo profiles of size-fractionated ectosomes derived from three thyroid cancer cell lines [BCPAP (papillary), CAL-62 (anaplastic), and 8505C (anaplastic)] and evaluated their capacity to reprogram THP-1 macrophages toward a pro-tumoral state.

METODI

EVs were isolated from cell culture supernatants via differential centrifugation at 2,800 × g, 10,000 × g (large microvesicles/oncosomes), and 100,000 × g (smallest microvesicles). Structural lineage markers and immunomodulatory molecules (CD80, CD163, TGF-β, GDF15), and HMGB1 were characterized using Western blot, qRT-PCR, flow cytometry, and confocal microscopy. Recipient THP-1 cells were treated with distinct EV fractions to assess activation and M2-like polarization.

RISULTATI

BCPAP cells yielded the lowest quantity and smallest size EVs which lacked significant immunomodulatory proteins. Conversely, aggressive anaplastic lines (CAL-62, 8505C) shed larger MVs reminiscent of large oncosomes, enriched in CD163 and TGF-β. While HMGB1 protein was undetectable across EVs from different cell lines—ruling out necrotic debris contamination—HMGB1 mRNA transcripts were selectively packaged inside the smallest vesicle fractions. Functionally, CAL-62 EVs strongly activated and polarized THP-1 cells into an immunosuppressive M2 phenotype (upregulated CD163 and TGFβ and down-regulated CD80). Remarkably, although GDF15 protein was absent in all native EVs, CAL-62 EVs induced robust de novo expression of GDF15 in recipient THP-1 macrophages.

CONCLUSIONI

Our findings demonstrate a distinct decoupling between stemness-associated structural traits and immunosuppressive cargo. Aggressive anaplastic thyroid cancer cells leverage large ectosomes to actively deliver transcriptomic blueprints and reprogram microenvironmental macrophages into autonomous sources of immunosuppressive factors.

A110

CUPRIZONE EXERTS MICROGLIA-MEDIATED NEUROTOXIC EFFECTS AND MODULATES AUTOPHAGY THROUGH G-QUADRUPLEXES IN NEURONAL CELLS.

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INTRODUZIONE

Demyelination is a hallmark of multiple sclerosis and several neurodegenerative diseases, in which altered central nervous system cell interactions and impaired autophagy contribute to pathology. Cuprizone (CPZ), a copper chelator widely used as an experimental demyelinating agent in animal models, has poorly defined cell-specific effects. In this study, we investigated the effects of CPZ on cells viability, microglial activation, neuronal–microglial interactions, and autophagy regulation in human neuronal (SH-SY5Y) and microglial (HMC3) cells, focusing on the role of G-quadruplex (G4) DNA structures.

METODI

CPZ effect on SH-SY5Y and HMC3 cell viability was assessed after 24 and 48h by trypan blue exclusion assay at different concentrations (1, 10, 100 μM). Microglial activation markers (CD11b, HLA-ABC, CD86, CD80, CD206 and HLA-DR), apoptosis/necrosis and G4 were evaluated by flow cytometry. Modulation of autophagy markers (BECN1 and LC3) was evaluated in SHSY5Y cells treated with CPZ (10 μM) and in the presence of quercetin (50nM) or metformin (1mM) by Western blot. Putative Quadruplex-Forming Sequences (PQFS) in the MAP1LC3A promoter were evaluated by QGRS Mapper.

RISULTATI

CPZ treatment did not directly affect neuronal or microglial cell viability, however, CM from CPZ-activated microglial cells, characterized by HLA-DR up-regulation, induced early apoptosis in SH-SY5Y cells, suggesting an indirect microglia-mediated neurotoxic effect. CPZ selectively impaired neuronal autophagy by reducing LC3II and LC3-II/LC3-I ratio without affecting BECN1 expression, no significant changes were detected in HMC3 cells. GQRS Mapper analysis identified PQFS within the LC3 promoter. Consistently, CPZ induced G4 unfolding and reduced LC3II expression in SH-SY5Y cells, similar effect was obtained with pyridostatin, a known G4 ligand. Moreover, co-treatment with neuroprotective agents, quercetin or metformin prevented CPZ-induced G4 unfolding and restored LC3 levels in SH-SY5Y cells.

CONCLUSIONI

Our data reveal a novel mechanism by which CPZ disrupts neuronal autophagy through G4 structures. Moreover, the protective effects of quercetin and metformin highlight the therapeutic potential of strategies aimed at preserving G4 stability and neuronal autophagy in demyelinating and neurodegenerative diseases.

A111

SEX-DEPENDENT MODULATION OF PARASITE CONTROL AND INFLAMMATORY MEDIATORS IN LEISHMANIA INFANTUM-INFECTED MACROPHAGES

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INTRODUZIONE

Leishmaniases are neglected tropical diseases caused by Leishmania and transmitted to humans by sandflies. Higher prevalence and worse clinical outcomes of leishmaniasis are reported in adult men compared to women, consistent with the generally better outcome of infectious diseases observed in women. However, the pathogenic mechanisms underlying this sex bias in leishmaniasis remain unclear. Macrophages are the primary host cells for Leishmania amastigotes and display plasticity, ranging from pro-inflammatory M1 (parasite killing) to anti-inflammatory M2 (parasite survival). The aim of this study was to investigate sex-related differences in the immune response to L. infantum infection in unpolarized (M0), M1 and M2 monocyte-derived macrophages (MDM) from male and female donors.

METODI

Primary human MDM were differentiated from peripheral blood monocytes of healthy donors. M0, M1 (IFNγ+TNFα) and M2 (IL-4) macrophages were infected with L. infantum MHOM/TN/80/IPT1 or a tdTomato-expressing strain (MOI 1 or 10). Phagocytosis and killing were assessed through Guava® Muse® Cell Analyzer. Expression of 16 genes related to inflammation and oxidative stress was analysed by Real-Time PCR. TNFα, IL-10 and IL-6 levels in the supernatants were measured by ELISA.

RISULTATI

Infection rates were significantly lower in M1-polarized MDM compared to M0 and M2. Male and female MDM showed similar phagocytosis, but female MDM exhibited a greater reduction in infection at 24-48-72h post-infection, suggesting enhanced killing activity. M1 MDM were the most effective in controlling infection. Preliminary data from 2 males and 2 females showed an upregulation of CCL5, CCR5, and PTGES (Prostaglandin E Synthase) in M1 MDM, further increased by infection and with no sex differences. Lastly, TNFα levels were significantly higher in M1 MDM than M0 and greater in males. Upon infection, a modest TNFα increase was observed only in female M0 and M1 MDM.

CONCLUSIONI

These data highlight preliminary sex-related differences in macrophage responses to polarization and L. infantum infection, particularly in parasite control and cytokines production. This work provides novel insights into sex bias in leishmaniasis and highlights the importance of integrating sex as a biological variable in therapeutic strategies.

A112

CBX6 AS AN EPIGENETIC REGULATOR OF DIFFERENTIATION IN GLIOBLASTOMA

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INTRODUZIONE

Glioblastoma(GBM) is the most aggressive primary brain tumor, characterized by pronounced epigenetic plasticity and maintenance of a stem-like, therapy-resistant state.CBX6, one of the Chromobox Family Protein and a component of the canonical PRC1 complex, is consistently downregulated in GBM.Its reduced expression correlates with higher tumor grade and IDH wild-type status, suggesting a potential tumor-suppressive role. However, the functional contribution of CBX6 to GBM biology, and particularly its involvement in differentiation programs, remains poorly understood.

METODI

CBX6 expression was evaluated through in silico analyses (GEPIA2, CGGA, TCGA) and validated in GBM cell lines by RT-qPCR, Western blotting, and immunofluorescence. A CBX6-overexpressing U87-MG model was generated by lentiviral transduction. Cell viability, cycle progression, migration, temozolomide response, and clonogenicity were assessed. ATRA-induced differentiation was analyzed by evaluating NES, MAPT, and β-Tubulin III expression.

RISULTATI

CBX6 expression was significantly reduced in GBM, particularly in WHO grade IV and IDH wild-type tumors, and this downregulation was confirmed in GBM cell lines at both mRNA and protein levels. Although CBX6 overexpression in U87-MG cells did not affect viability, cell cycle, migration, or temozolomide sensitivity, it significantly reduced clonogenicity. During ATRA-induced differentiation, CBX6 expression progressively increased alongside decreased Nestin and increased MAPT levels. Consistently, CBX6-overexpressing cells showed reduced Nestin and enhanced β-Tubulin III expression, supporting a role for CBX6 in promoting GBM cell differentiation.

CONCLUSIONI

These findings identify CBX6 as a potential epigenetic regulator of cancer cell identity in GBM, whose loss may contribute to the maintenance of stem-like features and increased tumor aggressiveness. Ongoing studies aim to further elucidate the CBX6-dependent transcriptional and chromatin programs using RNA-seq and ATAC-seq approaches, to evaluate its role in promoting differentiation across multiple GBM models, and to assess its functional relevance in vivo and therapeutic potential for differentiation-based strategies.

A113

UNVEILING THE ROLE OF THE M6A MODIFICATION IN CELLULAR SENESCENCE AND ITS IMPLICATION IN CANCER

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INTRODUZIONE

Cellular senescence is a multifaced and irreversible biological process characterized by permanent cell cycle arrest accompanied by sustained metabolic activity. It functions as a fundamental tumor-suppressive mechanism, typically initiated by cellular stress, and oncogenic activation. This process is tightly regulated by the p53/p21 and p16/RB tumor suppressor pathways and is associated with the secretion of pro-inflammatory senescence-associated secretory phenotype (SASP) which profoundly alters the tissue microenvironment. Notably, senescence exhibits a dual role in cancer, exerting both beneficial and detrimental effects depending on the context. A novel compound, Inflachromene (ICM), has been shown to induce senescence-like phenotype via HMGB1/2 inhibition without SASP activation offering a model to study senescence independently of inflammation. Despite extensive characterization of senescence-associated pathways, the role of the epitrascriptomic regulation, in particular the RNA modification N6-methyladenosine (m6A) remains unexplored. This study aims to investigate the involvement of m6A machinery in cellular senescence.

METODI

Human fibroblast cell lines, including IMR90 and WI38 cells, were treated with ICM at different concentrations and time point to establish an in vitro model of senescence. Senescence induction was evaluated through morphological assessment, cell cycle analysis, #-galactosidase assay and by the analysis of canonical senescence markers including p16 and p21. In parallel, variation of the major m6A regulators, including writers (METTL3, METTL14), eraser (FTO, ALKBH5) and readers are being analyzed following ICM treatment.

RISULTATI

ICM treatment induced morphological alterations consistent with a senescence like-phenotype, including enlarged and flattened cell morphology, together with cell cycle arrest following treatment. Preliminary molecular analyses suggest a modulation of m6A-related factors during ICM-induced senescence, supporting a potential involvement of epitrascriptomic mechanism in this process.

CONCLUSIONI

Overall, this study may contribute to a better understanding of the role of m6A in the regulation of cellular senescence and may uncover promising strategies for senescence-associated disease.

A114

COMBINED ALK AND HDAC TARGETING REDUCES ALK+ ALCL SURVIVAL AND MIGRATION

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INTRODUZIONE

Anaplastic large cell lymphoma (ALCL) is a T-cell malignancy frequently driven by constitutive activation of anaplastic lymphoma kinase (ALK). Although the ALK inhibitor crizotinib shows significant clinical activity in ALK+ ALCL, the development of resistance remains a major therapeutic challenge. Epigenetic dysregulation contributes to lymphoma cell survival and adaptation to targeted therapies, providing a rationale for combination approaches. We investigated if Belinostat, a pan-histone deacetylase inhibitor approved for relapsed or refractory peripheral T-cell lymphomas, enhance the antitumor activity of ALK inhibition.

METODI

ALK+ ALCL cell lines (SUPM2 and L82) and PDX-derived cells were treated with crizotinib and belinostat, alone or in combination. Cell viability was evaluated in monoculture and in co-culture with stromal cells to assess microenvironment-mediated drug protection. Cell death was quantified by flow cytometry after 72 h of treatment. Migratory capacity was assessed using transwell migration assays following drug pre-treatment, and migrated cells were quantified by crystal violet staining. Combination effects were compared with single-agent treatments.

RISULTATI

Stromal cells significantly reduced crizotinib-induced cell death, confirming a protective microenvironmental effect. Belinostat showed limited cytotoxic activity in co-culture, while the combination induced higher levels of cell death than single agents across all models. These findings indicate that HDAC inhibition enhances ALK-targeted therapy and partially counteracts stromal-mediated resistance. Moreover, migration assays revealed a pro-migratory effect of crizotinib in SUPM2 and L82 cells. Belinostat effectively counteracted this response, and combined treatment reduced migration compared to the control, suggesting a strong inhibitory effect on ALK+ ALCL cell motility.

CONCLUSIONI

Combined ALK and HDAC inhibition mediate a synergistic antitumor effect in ALK+ ALCL models. Beyond enhancing cell death, this strategy mitigates stromal-mediated protection and counteracts crizotinib-induced pro-migratory effects. These findings support dual ALK and epigenetic targeting as a promising approach to improve treatment efficacy and limit ALCL cell dissemination.

A115

THE ROLE OF EPHA2-POSITIVE EXTRACELLULAR VESICLES IN GLIOBLASTOMA VASCULARIZATION: INHIBITORY EFFECTS OF THE A40S APTAMER

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INTRODUZIONE

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults and is characterized by extensive vascularization, which sustains tumor growth, invasion, and therapeutic resistance. Increasing evidence suggests that extracellular vesicles (EVs) released by glioblastoma stem cells (GSCs) actively contribute to the establishment of a pro-angiogenic tumor microenvironment. Among the molecules involved in these processes, the receptor tyrosine kinase EphA2 has emerged as a key regulator of GBM progression, vascular mimicry, and intercellular communication. In this study, we investigated the contribution of EphA2-positive EVs released by GSCs to glioblastoma vascularization and evaluated the ability of the EphA2-targeting aptamer A40s to interfere with these pro-angiogenic mechanisms.

METODI

EVs were isolated from GSC cultures and characterized by Nanoparticle Tracking Analysis and Western blot. Tube formation EVs were isolated from GSC cultures and characterized by Nanoparticle Tracking Analysis and Western blot. Tube formation assays were performed to investigate the role of EphA2 in vascular network formation, assess the pro-angiogenic activity of GSC-derived EphA2-positive EVs, and evaluate the effects of A40s-mediated EphA2 targeting EV-driven angiogenesis. In parallel, A40s was employed to selectively recognize EphA2-positive EVs. The translational relevance of this approach was further assessed using EVs isolated from sera of GBM patients.

RISULTATI

Functional assays demonstrated that EphA2 contributes to vascular network formation and that GSC-derived EphA2-positive EVs promote angiogenic responses. Treatment with the A40s aptamer significantly reduced EV-mediated angiogenic features, supporting the involvement of EphA2 signaling in these processes. Furthermore, A40s selectively recognized EphA2-positive EVs derived from both GSC cultures and GBM patient sera.

CONCLUSIONI

Our findings identify EphA2-positive EVs as important mediators of glioblastoma vascularization and demonstrate that A40s can interfere with their pro-angiogenic activity. These results support the development of A40s-based strategies for targeting EV-mediated EphA2 signaling and highlight the potential of EphA2-positive EVs as clinically relevant biomarkers in GBM.

A116

NANOPLASTICS RESHAPE INTERCELLULAR COMMUNICATION IN THE BONE MARROW NICHE THROUGH EV AND TNT-MEDIATED PATHWAYS

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INTRODUZIONE

Nanoplastics (NPs) are emerging environmental contaminants capable of interacting with biological systems, yet their effects on intercellular communication in the bone marrow (BM) microenvironment remains unclear. This study investigated the impact of polystyrene NPs on extracellular vesicle (EV) biogenesis, cargo, and function using in vitro models of multiple myeloma (MM) and stromal cells, supported by ex vivo murine bone and human BM aspirates.

METODI

The OPM2, HS5, hTERT (stromal) and MC3T-E1 cell lines were used for the in vitro studies. Bone marrow aspirate and bone tissue were used for ex vivo approach. Cells were exposed to NPs (1-200 ug/ml) for 24-48 hours. NPs uptake were evaluated by flow-citometry, fluorescence microscopy and IVIS Spectrum imaging system. Cell viability and proliferation were assessed using the MTT assay. ROS level was determined by DCF assay. mRNA and miRNA levels were analyzed using qPCR. The profile of the EVs was determined by NTA, spectral flow-citometry, and cryo-EM.

RISULTATI

NPs were efficiently internalized by both malignant and stromal cells, inducing oxidative stress and reducing cell viability. In addition, hTERT stromal cells exhibited intercellular transfer of NPs through tunneling nanotube-like structures, suggesting an additional mechanism for nanoplastic dissemination within the BM microenvironment. NP exposure altered EV biogenesis, leading to increased vesicle release, reduced size, and the incorporation of NPs into a subset of EVs. Additionally, EVs from NP-exposed MM cells showed decreased total miRNA content and modulation of miRNA sorting-related proteins. Among the analyzed miRNAs, miR-505, a candidate linked to osteogenic regulation, was reduced in both cells and EVs following NP exposure. Functionally, EVs from control MM cells downregulated osteogenic markers in pre-osteoblasts, whereas EVs from NP-exposed cells showed reduced regulatory effects.

CONCLUSIONI

These findings demonstrate that NP exposure modifies EV-mediated signaling under experimental conditions and highlight TNT-mediated NP transfer as a potential additional route of communication within the BM niche, providing mechanistic insight into nanoplastic interference with BM cellular communication. Our study underscores the potential of environmental nanoplastic exposure to reshape cellular communication networks within the BM niche, with possible consequences for bone homeostasis and hematologic malignancies.

A117

FUNCTIONAL POLARIZATION OF TUMOR-ASSOCIATED γδ T CELLS IN MMR-DEFICIENT COLORECTAL CANCER

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INTRODUZIONE

γδ T cells represent a distinct subset of T lymphocytes that bridge innate and adaptive immunity and are increasingly recognized as important players within the tumor microenvironment. However, their functional heterogeneity in colorectal cancer (CRC) and its relationship with mismatch repair (MMR) status remain poorly characterized.

METODI

Single-cell RNA sequencing datasets from CRC patients (GSE178341 and GSE205506) were integrated and analyzed to characterize tumor-associated γδ T cells. γδ T cells were identified through reference-based cell mapping and subsequently subclustered based on transcriptional profiles. Functional programs were evaluated using canonical marker genes and module score analysis, including cytotoxic, inflammatory, exhaustion, activation, memory-like and cycling programs. Comparative analyses were performed across normal versus tumor tissues, MMR-proficient (MMRp) versus MMR-deficient (MMRd) tumors, untreated versus immune checkpoint inhibitor (ICI)-treated samples.

RISULTATI

Transcriptional profiling identified heterogeneous γδ T-cell populations displaying activated, cytotoxic, inflammatory, memory-like and proliferative programs. Comparative analyses revealed differences in γδ T-cell cluster distribution between MMRd and MMRp cancers, with relative differences in effector-like populations across MMR groups. Module score analysis further showed increased cytotoxic and exhaustion-related programs together with reduced memory-like signatures in MMRd tumors compared with MMRp tumors. Cluster-specific analyses indicated that these functional differences were particularly evident within cytotoxic and inflammatory γδ T-cell populations. Correlation analysis additionally supported the existence of coordinated effector-like γδ T-cell states across tumor-associated γδ T cells, characterized by cytotoxic, inflammatory and exhaustion-associated programs.

CONCLUSIONI

Our findings reveal substantial functional heterogeneity among CRC-associated γδ T cells and suggest an association between MMR deficiency and γδ T-cell polarization toward cytotoxic and chronically activated states. These results provide insight into the immune landscape of MMRd CRC and support a potential contribution of γδ T cells to anti-tumor immune responses.

A118

ANTI-PLASMODIAL AND ANTI-INFLAMMATORY ACTIVITY OF SYZYGIUM AROMATICUM AND ITS COMPONENTS

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INTRODUZIONE

Malaria is one of the most impactful parasitic diseases in tropical countries, causing over 600000 deaths annually, particularly in children under five years of age. Among human malaria parasites, Plasmodium falciparum is the most dangerous species and is responsible for severe cases and deaths. Disease pathogenesis and severity is associated with cytoadherence of infected red blood cells to the microvasculature and an excessive inflammatory response. The emergence of drug-resistant parasites highlights the need for new drugs able to both kill the parasite and reduce pathogenesis. Plant extracts represent a valuable source of bioactive compounds. Here, we evaluated the anti-plasmodial activity of Syzygium aromaticum and selected constituents, including α-humulene, eugenyl acetate, and β-caryophyllene, against Plasmodium falciparum.

METODI

Parasite viability was assessed by pLDH assay in chloroquine-sensitive (D10) and -resistant (W2) strains, and IC50 values were calculated for both the asexual stages and gametocytes, the stage responsible for transmission. Cytotoxicity on human cells was determined by MTT assay, and selectivity index (SI) calculated. Compounds were also tested in combination, and interactions were analysed by isobologram analysis. Anti-inflammatory activity was evaluated in PMA-differentiated THP-1 macrophages by measuring cytokine production by ELISA.

RISULTATI

The aqueous extract of S. aromaticum showed IC50 values of 27 and 20 μg/mL against D10 (Chloroquine-sensitive) and W2 (Chloroquine-resistant) strains, respectively. Low activity was observed for gametocytes. Among the isolated components, α-humulene and β-caryophyllene exhibited higher potency (IC50 5–6 μg/mL in both strains) with low cytotoxicity (SI >10), whereas eugenyl acetate was less active and more toxic. Combination studies indicated an additive effect between α-humulene and β-caryophyllene. α-Humulene also inhibited LPS-induced IL-6 and IL-8 (but not TNF-α) production.

CONCLUSIONI

S. aromaticum and its components, particularly α-humulene and β-caryophyllene, showed promising anti-plasmodial activity associated with moderate anti-inflammatory effects.

A119

DEVELOPMENT OF AN IN VITRO GLAUCOMA MODEL: MOLECULAR INSIGHTS INTO GLIAL CELL LINES

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INTRODUZIONE

Glaucoma is a chronic neuropathy characterized by progressive degeneration of retinal ganglion cells and loss of optic nerve fibers. It represents the leading cause of irreversible blindness worldwide and the second most common cause in Europe (Stuart KV et al., 2025). While elevated intraocular pressure (IOP) is the primary and only modifiable risk factor, current therapies focusing on IOP reduction are often insufficient to halt disease progression (Jayaram H et al., 2023). Recent evidence points to neuroinflammation as a pivotal driver of the pathology, with the NF-κB pathway acting as a master transcriptional regulator of glaucomatous neurodegeneration. However, the specific mechanisms by which retinal glial cells initiate and sustain this dysfunction remain unresolved (Veglianti F et al., 2026). Therefore, the main aim of this study is to develop an in vitro model that mimics glaucomatous conditions to assess the individual and synergistic contributions of macroglia and microglia in the context of neuroinflammation.

METODI

We simulated hydrostatic pressure using IVTech technology (Live Flow + Live PA) in glial cell lines mimicking the glaucomatous environment. The cell lines were cultured both in monoculture and in a millifluidic co-culture system under stress conditions to mimic the effects occurring during glaucoma pathogenesis. Western blotting analysis was performed to investigate the involvement of inflammatory and survival signaling pathways in glial cell lines under pressure-induced stress.

RISULTATI

Live Flow + Live PA technology in cultured glial cells induced increased cellular distress compared to controls. This resulted in modulation of key molecular pathways, including NF-κB, AKT, and STAT3. Differential responses were observed across microglial and macroglial lines, as well as between monoculture and co-culture conditions

CONCLUSIONI

The activation of NF-κB, AKT, and STAT3 pathways confirms that this in vitro bioreactor system potentially replicates key pathophysiological hallmarks of glaucoma, specifically neuroinflammation and reactive gliosis. Therefore, this technology provides a robust platform for simulating complex disease mechanisms in vitro, offering a valuable tool for drug screening while significantly contributing to the reduction of in vivo experimental models.

A120

MULTI-OMIC ANALYSIS LINKS BAZ1B ACTIVITY TO PI3K/MTOR SIGNALING IN ERα-POSITIVE BREAST CANCER

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INTRODUZIONE

Endocrine therapy resistance remains a major clinical challenge in ERα-positive (ERα#) breast cancer (BC). In this context, PI3K pathway alterations can promote signaling hyperactivation and reduced sensitivity to endocrine-based therapies, supporting the use of PI3Kα inhibition in selected resistant tumors. Based on previous evidence that BAZ1B silencing modulates PI3K/mTOR signaling, this study investigated how BAZ1B influences this cascade and may expose vulnerabilities in resistant phenotypes.

METODI

We investigated the BAZ1B-dependent transcriptional regulation of PI3K-associated genes by combining chromatin immunoprecipitation (ChIP), mass spectrometry-based interaction proteomics, and phosphoproteomics. The translational impact of this epigenetic axis was subsequently challenged in vitro by coupling siRNA-mediated BAZ1B knockdown with the PI3Kα inhibitor. Phenotypic consequences of this combinatorial approach were profiled through functional survival and apoptosis assays in a panel of ERα# breast cancer cell lines that included both therapy-sensitive and endocrine/palbociclib-resistant models.

RISULTATI

BAZ1B-associated networks were characterized by IP-MS in MCF7 cells and compared with previously defined Dot1L-, menin- and ERα-associated interactomes, identifying a BAZ1B-centered interactome enriched for RNA processing, chromatin remodeling, DNA repair, cell architecture and PI3K/mTOR-related signaling modules. Phosphoproteomic profiling following BAZ1B knockdown revealed signaling changes consistent with modulation of PI3K/mTOR-related pathway activity. Functional experiments suggest that BAZ1B depletion may enhance alpelisib response, affecting proliferation and viability in therapy-sensitive and endocrine/palbociclib-resistant ERα# models.

CONCLUSIONI

These preliminary data extend our previous work by linking BAZ1B-dependent chromatin networks to PI3K/mTOR pathway activity. Our findings support further testing of BAZ1B silencing combined with a PI3Kα inhibitor in therapy-sensitive and resistant ERα-positive BC models, particularly in the context of PI3K pathway activation or PIK3CA alterations.

A121

PATIENT-DERIVED LIVER ORGANOIDS AS A PLATFORM FOR DISEASE MODELLING

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INTRODUZIONE

Primary liver cancer (PLC), including hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA), are associated with poor outcomes, and reliable predictive biomarkers and validated therapeutic strategies are urgently needed. In this regard, accurate in vitro models are necessary to better understand the molecular and cellular processes underlying PLC development and to enable high-throughput assessment of treatments efficacy.
This study aimed to evaluate patient-derived organoids treated with different agents, by screening a library of substances that experimentally affect tumor cell viability, to identify candidate therapeutic compounds against PLC.

METODI

To develop liver organoids, tumor and non-tumor biopsies were minced and enzymatically digested into small cell clusters, which were subsequently embedded in Matrigel. Following morphological and molecular characterization by immunofluorescence and qPCR, the resulting organoid cultures were used as a disease modelling platform to recapitulate patient-specific liver pathology in vitro.

RISULTATI

We developed and characterized tumor and non-tumor organoids, evaluating morphological features and confirming typical markers. Subsequently, to validate the reliability of this 3D model, we tested their sensitivity to sorafenib (HCC) and gemcitabine+cisplatin (iCCA), demonstrating the efficacy of these drugs.
In the context of disease modelling, to better mimic the tumor microenvironment, we co-cultured PLC-organoids with patient-derived immune cells, evaluating the antibody-dependent cell cytotoxicity and resulting in a limited efficiency in organoid reforming after treatments.
In parallel, we induced carcinogenic transformation in normal organoids, using N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), to evaluate the effect of 1-Piperidinepropionic acid, a SerpinB3 inhibitor, observing reduced proliferation and increased apoptotic cell death.
Furthermore, we generated functional multi-cellular liver non-tumor organoids with macrophages and stellate cells, inducing cell differentiation and treating them with fatty acids, to model steatosis and fibrosis.

CONCLUSIONI

A well-defined in vitro model of PLC was developed and characterized, enabling the investigation of various substances as potential novel therapeutic strategies.

A122

γδ T CELL REMODELING AND IMMUNE CHECKPOINT SIGNATURES DURING PD-1 BLOCKADE IN NON-SMALL CELL LUNG CANCER

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INTRODUZIONE

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all diagnosed cases. Despite significant advances in targeted therapies and immunotherapy, the prognosis of patients with advanced NSCLC remains poor, underscoring the urgent need for a deeper understanding of tumor immune dynamics and for the identification of reliable biomarkers associated with therapeutic response and resistance mechanisms. γδ T cells contribute to antitumor immunity and represent potential biomarkers in NSCLC, but their modulation during immune checkpoint blockade remains poorly defined. We investigated longitudinal changes in peripheral Vδ1 and Vδ2 γδ T cell subsets in NSCLC patients treated with pembrolizumab and assessed their association with immune checkpoint pathways and survival.

METODI

Peripheral blood samples collected before and during treatment were analyzed by conventional flow cytometry and computational analysis. Soluble immune checkpoint molecules were quantified by Luminex assay, and transcriptomic analyses were performed in the TCGA-LUAD cohort.

RISULTATI

Pembrolizumab induced expansion of circulating Vδ1 and Vδ2 γδ T cells, accompanied by selective modulation of inhibitory receptors, including reduced PD-1 expression on Vδ1 cells and reduced TIM-3 expression on Vδ2 cells. Unsupervised FlowCT analysis identified an NKG2A+ TIM-3+ Vδ2+ population that significantly expanded during therapy. Increased frequencies of TIM-3+ Vδ2 T cells were associated with prolonged overall survival, whereas higher frequencies of PD-1+ or TIM-3+ Vδ1 T cells correlated with poorer outcomes. Patients also displayed marked dysregulation of soluble immune checkpoint molecules. Transcriptomic analyses showed that γδ T-cell signatures, particularly a Vδ2-like signature, were associated with favorable survival and mitigated the adverse prognostic impact of high PDCD1 and HAVCR2 expression.

CONCLUSIONI

These findings support a potential protective role of γδ T cells, particularly the Vδ2-like compartment, in shaping antitumor immunity and modulating the prognostic impact of immune checkpoint pathways. These results suggest that γδ T-cell–associated signatures may represent promising biomarkers for immune stratification and potentially novel targets for combinatorial immunotherapeutic approaches in NSCLC.

B001

ANTI-MDA5 AUTOANTIBODIES DRIVE PRO-FIBROTIC RESPONSES IN HUMAN LUNG FIBROBLASTS

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INTRODUZIONE

Anti-MDA5 autoantibodies identify a dermatomyositis subset at high risk of rapidly progressive interstitial lung disease, but their direct effects on lung fibroblasts remain unclear. This study investigated whether patient-derived anti-MDA5 antibodies and MDA5/anti-MDA5 immune complexes modulate IMR-90 fibroblast behaviour and profibrotic responses.

METODI

Recombinant human MDA5 was produced in HEK293F cells and used as an affinity matrix to purify polyclonal anti-MDA5 antibodies from patient plasma, followed by MabSelect chromatography and isotype characterization. IMR-90 human lung fibroblasts were treated with MDA5, anti-MDA5 antibodies, or MDA5/anti-MDA5 immune complexes. Cell behaviour was monitored for 5 days by Real-Time Cell Analysis (RTCA). Metabolic activity was assessed by MTT assay, cell number by direct counting, gene expression by RT-qPCR, and secreted interferons/pro-collagen by ELISA.

RISULTATI

Purified antibodies were mainly IgG1, with both kappa and lambda light chains. RTCA showed that all treatments were tolerated, but MDA5/anti-MDA5 immune complexes induced the highest normalized cell index at 121 h, with an approximately 25% increase versus control ($p=0.0182$), and reduced doubling time from 54.41±0.52 h to 46.76±0.33 h ($p=0.0182$). Complement inactivation abolished the impedance response. MTT analysis showed no significant cytotoxicity; anti-MDA5 antibodies showed only a borderline reduction in metabolic activity ($p=0.0606$). Direct cell counting confirmed increased fibroblast proliferation after immune-complex treatment ($p=0.0123$). RT-qPCR plots indicated significant modulation of innate immune and fibrotic markers, including TLR2, TLR7, IFN-related genes and ET-1. ELISA data showed increased secretion of IFN-alpha, IFN-beta, IFN-gamma and pro-collagen mainly in anti-MDA5 antibody-treated cells, while immune-complex exposure showed a distinct secretory profile.

CONCLUSIONI

Patient-derived anti-MDA5 antibodies affect human lung fibroblast behavior. In particular, MDA5/anti-MDA5 immune complexes enhance fibroblast impedance and proliferation through a complement-sensitive mechanism, supporting a direct pathogenic contribution to lung tissue remodeling in anti-MDA5-associated disease.

B002

CBX2 AS A POTENTIAL TARGET FOR THERAPEUTIC INTERVENTION IN CRC

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INTRODUZIONE

Colorectal cancer (CRC) is driven by the accumulation of genetic and epigenetic mutation with disrupted epigenetic control play a key role in CRC onset and progression. These alterations offer new avenues for potential therapies. Among the epigenetic targets, CBX proteins (CBXs) have emerged as promising candidates. Acting as epigenetic readers, CBXs recognize histone modifications like H3K27me3 and H3K9me3, thereby influencing chromatin conformation and gene expression. Many CBXs are dysregulated in cancer, however CBX2 specifically linked to poor prognosis in CRC. Despite this, the exact molecular role of CBX2 in CRC remains elusive.

METODI

This study investigated the involvement of CBX2 and its domains in CRC progression. CBX2 expression was evaluated in primary CRC samples and compared with normal tissue. To assess its functional role, a reverse genetics approach was employed by silencing CBX2 in CRC cells. Cell growth, cell death, and migratory capacity were then analyzed following CBX2 depletion. To identify the molecular mechanisms underlying the observed phenotype, transcriptome-wide analysis was performed on CBX2-silenced CRC cells.

RISULTATI

CBX2 was found to be overexpressed in primary CRC samples compared with normal tissue. CBX2 silencing impaired CRC cell growth, induced cell death, and reduced cell migration, thereby limiting invasive potential. Transcriptome-wide analysis revealed the downregulation of key gene sets involved in CRC survival pathways, including eEF2 targets and the TGFβ pathway. In contrast, genes associated with apoptosis were upregulated, indicating a shift toward pro-apoptotic signaling following CBX2 depletion.

CONCLUSIONI

Our findings underscore the critical role of CBX2 in CRC carcinogenesis. CBX2 overexpression promotes tumor growth and invasiveness, and silencing of CBX2 effectively suppresses CRC cell survival, presenting a promising therapeutic strategy.

B003

C-TYPE LECTIN RECEPTORS CLEC4A AND CLEC12A ON PERIPHERAL MYELOID CELLS ARE INVERSELY ASSOCIATED WITH CANCER PATIENTS PROGNOSIS

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INTRODUZIONE

C-type lectins are immunoregulatory receptors implicated in tumor progression, poor prognosis, and immune suppression. We recently identified a cluster of C-type lectins associated with survival in glioblastoma patients. However, their role in cancer remains poorly understood. In this study, we aimed to investigate the expression of C-type lectins on myeloid cells and evaluate their potential as immune biomarkers in cancer patients.

METODI

Single-cell RNA sequencing data from the TISCH2 database were analyzed to assess the distribution of C-type lectins across immune cell subsets in different solid tumors. The expression of CLEC4A and CLEC12A on circulating myeloid cells (CD45+ cells, monocytes, LOX1+ PMN-MDSCs, and M-MDSCs) was evaluated by multiparametric flow cytometry in peripheral blood samples from 70 cancer patients with various solid tumors and 6 healthy donors. Statistical comparisons were performed using the unpaired Student's t-test. Overall survival was analyzed using the Kaplan–Meier method and log-rank test.

RISULTATI

scRNA-seq analysis on TISCH2 revealed that both C-type lectins are expressed in all cancer histotype analyzed in association with myeloid cells, particularly monocytes and macrophages. C-type lectins were analyzed by flow-cytometry to validate bioinformatic analysis on PBMCs of HD and cancer patients. CLEC4A and CLEC12A were significantly upregulated in cancer patients on circulating immune cells, particularly on M-MDSCs, compared to HD. To evaluate the prognostic significance of C-type lectins, cancer patients were divided into two groups based on the median expression of CLEC4A and CLEC12A on the different myeloid subsets. Kaplan-Meier survival analysis revealed that high expression levels of CLEC4A and CLEC12A on circulating immune cells (both total CD45+ and M-MDSCs) were significantly associated with poor overall-survival. Interestingly, total CD45+ cells and M-MDSC did not show a correlation with prognosis.

CONCLUSIONI

CLEC4A+ and CLEC12A+ M-MDSCs may be exploited as potential circulating biomarkers of prognosis in cancer patients.

B004

FERRITIN NANOPARTICLES AS STRATEGIC DELIVERY TOOLS FOR HUMAN ORGANOTYPIC BRAIN SLICES: A PROOF-OF-CONCEPT STUDY

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INTRODUZIONE

Glioblastoma (GB) remains one of the most aggressive primary brain tumors despite multimodal treatment approaches. Although microRNA (miRNA)-based replacement therapies hold considerable therapeutic potential, their clinical application is hindered by poor stability, limited tissue penetration, and insufficient tumor-selective delivery. The transferrin receptor CD71, encoded by TFRC, is a key mediator of iron uptake and is highly expressed in metabolically active GB cells, making it a potential endogenous entry route for targeted delivery.

METODI

To characterize TFRC distribution within GB tissue, we integrated single-cell reference atlases with spatial transcriptomic analyses. CD71 protein expression was evaluated by immunohistochemistry, while miR-139-5p localization was assessed using miRNAscope in paired tumor and peritumoral regions. miRNA delivery was performed using an engineered humanized ferritin nanocage derived from Archaeoglobus fulgidus and loaded with a PAMAM dendrimer (hFt-NP). Nanoparticle uptake, tissue penetration, miR-139-5p restoration, and biological effects were investigated in patient-derived GB cultures and organotypic human cultures (OHCs).

RISULTATI

TFRC expression was associated with malignant astrocytic, hypoxic, and inflammatory transcriptional programs, with TFRC-high tumor cells preferentially localized within myeloid-rich niches. At the protein level, CD71 was markedly enriched in tumor regions, whereas miR-139-5p expression was predominantly retained in peritumoral areas. hFt-NPs efficiently penetrated OHCs, displayed CD71-associated uptake, restored miR-139-5p levels, reduced Ki-67 positivity, and promoted progressive cell-cycle arrest.

CONCLUSIONI

Our findings identify TFRC/CD71-high niches as biologically relevant targets for ferritin-mediated miRNA delivery in GB. In addition, they establish patient-derived organotypic human cultures (OHCs) as a clinically relevant ex vivo platform for studying nanoparticle penetration, targeted delivery, and therapeutic activity within preserved tumor microenvironmental contexts.

B005

IMMUNE CHECKPOINT EXPRESSION IN γδ T CELLS REFLECTS STRUCTURED REGULATORY CIRCUITS RATHER THAN A LINEAR EXHAUSTION TRAJECTORY

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INTRODUZIONE

Immune checkpoint receptors (ICRs) are commonly interpreted as hallmarks of T-cell exhaustion, yet their expression may also reflect physiological activation and differentiation programs. While this paradigm has been extensively studied in CD8 αβ T cells, the regulatory framework of ICR expression in human γδ T cells remains poorly defined.

METODI

Here, we investigated the dynamics of PD-1, TIM-3, LAG-3, TIGIT, and CTLA-4 expression across αβ and γδ T-cell subsets following both polyclonal activation and pharmacologic Vδ2 expansion with zoledronic acid (ZA), and in tumor-infiltrating lymphocytes (TILs), analyzed by single-cell transcriptomics before and after immune checkpoint blockade (ICB) therapy.

RISULTATI

Polyclonal stimulation induced broad, time-dependent upregulation of multiple ICRs across lineages. In contrast, ZA-driven Vδ2 expansion generated a selective hierarchy characterized by sustained TIM-3 expression, transient PD-1 induction and limited modulation of TIGIT and CTLA-4. Differentiation analyses revealed structured, subset-specific patterns: TIM-3, LAG-3, and CTLA-4 were enriched in naive γδ T cells, TIGIT preferentially marked effector memory populations, and PD-1 displayed an intermediate distribution.
Integration with a single-cell atlas of tumor-infiltrating γδ and CD8 T cells confirmed lineage-specific checkpoint programs. Whereas CD8 T cells exhibited progressive ICRs upregulation along differentiation trajectories, consistent with exhaustion-associated states, γδ T cells displayed heterogeneous, state-dependent expression profiles. In basal cell carcinoma and melanoma samples, ICB therapy increased T-cell numbers but did not uniformly reverse inhibitory signaling; instead, compensatory upregulation of alternative checkpoints, particularly TIGIT and LAG-3, was observed.

CONCLUSIONI

Collectively, these findings demonstrate that ICR expression in γδ T cells reflects activation-coupled regulatory circuits rather than a canonical exhaustion program. Recognition of these lineage-specific checkpoint hierarchies, with TIM-3 emerging as a central node, may inform combinatorial immunotherapeutic strategies incorporating γδ T cells.

B006

THE MITOCHONDRIAL PEPTIDE HUMANIN AS A PROTECTIVE BIOMARKER OF ENDOTHELIAL FUNCTION IN RHEUMATOID ARTHRITIS

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INTRODUZIONE

Rheumatoid arthritis (RA) is associated with an excess of cardiovascular risk, largely mediated by endothelial dysfunction (ED), which cannot be explained by traditional risk factors alone. Humanin (HN), a mitochondrial-derived peptide with known cytoprotective and antiapoptotic activity on the endothelium, may represent a protective mediator. This study evaluated the relationship between circulating HN, non-coding RNAs (lncRNA GAS5, miR-21, miR-103), and ED in patients with RA.

METODI

Ninety patients with RA were enrolled (EDRA substudy, NCT02341066) and stratified by cardiovascular risk using SCORE2-EULAR. Peripheral ED was assessed by flow-mediated pulse amplitude tonometry (EndoPAT), with Ln-RHI <0.51 considered indicative of dysfunction. HN was measured by ELISA; GAS5, miR-21, and miR-103 were quantified by qPCR in plasma and exosomes. Multivariate logistic regression analyses (adjusted for age, sex, and SCORE2-EULAR), ROC curves, and Kaplan–Meier survival analyses were performed.

RISULTATI

Higher HN levels were independently associated with better endothelial function (OR=0.98; p=0.02). ROC analysis indicated a moderate predictive value for ED (AUC=0.68; cutoff 124.44 pg/mL). Kaplan–Meier analysis showed significantly better survival in patients with higher HN (log-rank p<0.0001). GAS5, miR-21, and miR-103 were not significantly associated with ED.

CONCLUSIONI

Circulating HN is proposed as a potential protective biomarker of endothelial function and a prognostic biomarker in RA, supporting a role for the mitochondrial axis in inflammatory vasculopathy. The contribution of non-coding RNAs requires confirmation in larger cohorts.

B007

MACROPHAGE-DERIVED IRON SHAPES A PRO-TUMORIGENIC MICROENVIRONMENT IN CHOLANGIOCARCINOMA

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INTRODUZIONE

Cholangiocarcinoma (CCA) is a highly aggressive biliary tract malignancy characterized by poor prognosis and a tumor microenvironment (TME) enriched in tumor-associated macrophages (TAMs). Our previous studies showed that altered iron metabolism correlates with faster tumor growth and reduced survival in CCA patients, suggesting a role for macrophage-derived iron in tumor progression. To investigate how TAM iron export shapes the CCA microenvironment, we combined in vivo and in vitro approaches.

METODI

In vivo, CCA was induced in mice with myeloid-specific ferroportin deletion (Fpn-KO) and wild-type (WT) littermates by hydrodynamic tail vein injection of AKT and YAP oncogenes. Tumor development and stromal features were evaluated at 2, 3, and 4 weeks by CK19 immunohistochemistry and CD31/collagen I immunofluorescence. TME composition was further analyzed by flow cytometry at 3 weeks.
In parallel, a 3D bioprinted model was developed using murine CCA cells (SB1) co-cultured with peritoneal macrophages from WT or Fpn-KO mice in a hyaluronic acid-gelatin hydrogel (HA-GEL). Cell viability, proliferation, macrophage infiltration, and ECM remodeling markers (CHI3L1, vimentin, α-SMA) were assessed by immunofluorescence, while cytokine secretion (TNF-α, IL-1β, IL-6, CXCL2) was measured by ELISA. Tumor cells and macrophages were molecularly characterized by qRT-PCR following bead-based separation.

RISULTATI

In vivo, WT mice developed earlier and more aggressive tumors than Fpn-KO mice. At 3 weeks, CK19+ lesions were smaller in KO animals, whereas WT tumors showed increased vascularization and collagen I deposition, indicative of an activated stroma. Flow cytometry revealed a reduced immune compartment and lower tumor cell abundance in KO mice. Although both groups displayed extensive tumor burden at week 4, stromal activation remained more pronounced in WT animals.
In the 3D model, SB1 cells remained viable and proliferative. WT macrophages infiltrated the hydrogel more efficiently and promoted ECM remodeling, with increased CHI3L1, vimentin, and α-SMA expression. In contrast, Fpn-KO macrophages displayed a pro-inflammatory M1-like phenotype, characterized by higher TNF-α, IL-1β, IL-6, and CXCL2 secretion, and induced a less aggressive tumor cell phenotype.

CONCLUSIONI

These findings identify macrophage-derived iron as a key regulator of a tumor-supportive microenvironment in CCA and support TAM iron metabolism as a potential therapeutic target.

B008

THE COMBINATION OF NITROXOLINE WITH GEMCITABINE REDUCES METASTATIC BURDEN IN MOUSE MODELS OF PANCREATIC CANCER

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INTRODUZIONE

Pancreatic cancer (PC) is one of the most aggressive malignancies. Current therapies provide limited clinical benefit. Therefore, the identification of novel and more effective treatment strategies is urgently needed. In previous in vitro studies we demonstrated that nitroxoline, an antibiotic for urinary tract infections, interferes with several biological processes involved in PC progression, but evidence of its relevance in vivo is missing. Thus, we evaluated in preclinical models of pancreatic cancer the therapeutic potential of nitroxoline alone and in combination with gemcitabine

METODI

Human PC cell lines AsPC-1, PaTu-8902 and KP-4 were treated with nitroxoline and gemcitabine, alone or in combination. Cell viability and invasiveness were evaluated by MTT assay and xCELLigence Real-Time Cell Analyzer system, respectively. In vivo activity was assessed in NSG mouse xenografted with the three PC lines treated with nitroxoline (orally), gemcitabine (intraperitoneally), or their combination. Histological and immunohistochemical analyses were performed to evaluate necrosis, proliferation (Ki67), DNA damage (γ-H2AX), vascularization (CD31/CD105) and lung metastases. Statistical significance was assessed using Student's t-test and two-way ANOVA

RISULTATI

Nitroxoline significantly reduced PC cell viability and invasiveness in vitro, both as single agent and in combination with gemcitabine. In vivo, nitroxoline monotherapy was well tolerated and significantly inhibited tumor growth in all xenograft models tested. Also the combination treatment showed good tolerability and enhanced antitumor activity, with superior tumor growth inhibition, as compared with gemcitabine alone, in one xenograft model. Notably, the combination consistently reduced lung metastases across all models, showing a greater overall antimetastatic effect than gemcitabine alone. Histological and immunohistochemical analyses confirmed reduced proliferation, increased DNA damage and modulation of tumor vascularization in treated tumors

CONCLUSIONI

These findings identify nitroxoline as a promising drug repurposing candidate for pancreatic cancer treatment. In particular, its combination with gemcitabine may improve both antitumor and antimetastatic effects, supporting further clinical investigation in patients

B009

GDF-15 AND SST2 AS INDEPENDENT PREDICTORS OF LONG-TERM MORTALITY IN HOSPITALIZED OLDER ADULTS: A PROSPECTIVE COHORT STUDY

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INTRODUZIONE

Growth differentiation factor-15 (GDF-15) and soluble ST2 (sST2) are biomarkers increasingly recognized as indicators of systemic biological stress, inflammation, and aging-related processes. While their prognostic role has been extensively explored in cardiovascular disease, their relevance in hospitalized older adults with complex clinical profiles remains less well established.

METODI

In this prospective observational study, 966 patients aged ≥65 years from the Report-AGE cohort were included. Circulating GDF-15 and sST2 levels were measured at hospital admission. The primary outcome was all-cause mortality. Associations were assessed using Cox proportional hazards models, both univariable and multivariable, adjusting for demographic characteristics, frailty, comorbidity burden, and routine laboratory parameters. Incremental prognostic value was evaluated using likelihood ratio tests, Harrell's C-index, and time-dependent ROC analyses.

RISULTATI

Over a median follow-up of 10 years, 805 deaths occurred. Higher levels of both GDF-15 and sST2 were associated with increased mortality risk and remained independent predictors after multivariable adjustment (GDF-15: HR 1.32, 95% CI 1.17–1.49; sST2: HR 1.24, 95% CI 1.12–1.36; both $p<0.001$). The addition of each biomarker significantly improved model fit, with further gains observed when both were jointly considered. Discriminative ability improved modestly but consistently over time. In patients with cardiovascular disease, GDF-15 retained incremental prognostic value, while the contribution of sST2 was attenuated.

CONCLUSIONI

GDF-15 and sST2 provide independent prognostic information in hospitalized older adults. Their combined assessment may help capture the complex biological processes underlying vulnerability in this population and improve risk stratification beyond conventional clinical and laboratory markers.

B010

DISRUPTING GLIOBLASTOMA STEM CELL NICHES: TRANSLATIONAL DUAL TARGETING OF MET AND HYPOXIA

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INTRODUZIONE

Glioblastoma (GBM) is the most aggressive primary tumor of the central nervous system and remains invariably fatal. A major determinant of therapeutic failure and tumor recurrence is the persistence of GBM stem-like cells (GSCs). In particular, aberrant activation of the HGF/MET signaling axis and carbonic anhydrase IX (CA IX)-mediated pH regulation promote GSC stemness and cellular adaptation, thus fostering therapeutic resistance. Our hypothesis is that the simultaneous targeting of MET and CA IX could disrupt GSC maintenance and niche protection, leading to functional depletion of therapy-resistant GSCs and improved therapeutic vulnerability.

METODI

We established patient-derived GSC models of increasing complexity: neurosphere cultures, GSC-derived organoids, and orthotopic intracranial xenografts. Moreover, we develop and validate innovative hybrid small-molecule drug conjugates (SMDCs) designed to simultaneously target MET and CA IX, enhancing tumor penetration, efficacy, and therapeutic selectivity.

RISULTATI

The analysis of publicly available transcriptomic datasets demonstrates that high expression levels of MET and CA IX significantly correlate (alone and in combination) with poorer overall survival in GBM patients, giving prognostic relevance to these markers. We next validated MET and CA IX transcription and protein expression in three preclinical GSC models both in normoxic and hypoxic condition. The immunohistochemical analyses of tumor explants from orthotopic xenografts of GSCs into immunodeficient mice reveal robust MET expression and activation by phosphorylation together with strong CA IX expression within tumor tissues. Preliminary functional studies on neurospheres showed that the treatment with crizotinib (MET inhibitor) or SLC-0111 (CA IX inhibitor) resulted in a significant reduction of GSC proliferation. Notably, no significant antiproliferative effects were observed in MET-CAIX negative cells, indicating target-dependent activity of the inhibitors. Moreover, the combined treatment produced additive to synergistic effects on cell proliferation inhibition. Finally, a prototypical hybrid compounds (AA5719) effectively inhibits GSC proliferation, with IC_{50} values in the low micromolar range and with no significant effect on the control cells.

CONCLUSIONI

Collectively, these preliminary data strongly support the central hypothesis of the project and establish a solid experimental foundation for the proposed in-depth mechanistic and translational studies.

B011

ANTINEOPLASTIC ACTIVITY OF TYROSINE KINASE INHIBITORS CLM29 AND CLM3 IN PRIMARY ANAPLASTIC THYROID CANCER CELLS, FROM BIOPSY OR FINE NEEDLE ASPIRATION

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INTRODUZIONE

Anaplastic thyroid cancer (ATC) is a rare but highly aggressive endocrine malignancy, associated with a mean survival of approximately six months. Conventional treatment includes surgery, hyperfractionated accelerated external beam radiotherapy and chemotherapy, resulting in a median survival of about ten months. Thus, identifying an effective systemic therapy could represent a significant advancement in the management of this lethal disease. Moreover, the possibility of testing the sensitivity of primary ATC cell cultures (pATC) from individual patients to different drugs may help avoid the administration of ineffective therapies, thereby improving treatment efficacy.
In this study we aimed to evaluate the antineoplastic effect of two synthetized tyrosine kinase inhibitors (TKIs), CLM3 and CLM29 (“pyrazolo[3,4-d]pyrimidine” compounds) in pATC obtained both from biopsy (biop-pATC) and fine needle aspiration (FNA-pATC).

METODI

We tested the antiproliferative effect of CLM29 and CLM3 on biop-pATC and FNA-pATC established from 6 patients. We performed in vitro experiments using the following concentrations of the compounds: 5, 10, 30, 50 μM for CLM29, and 1, 5, 10, 30, 50, 100 μM for CLM3.

RISULTATI

In both biop-pATC and FNA-pATC we observed a significant reduction of proliferation compared to control after treatment with CLM29, and a slight but significant reduction after treatment with CLM3.
Subsequently, a dose-dependent increase in the rate of apoptosis was found in both biop-pATC and FNA-pATC, with no significant differences in sensitivity to CLM29 or CLM3.
Finally, a significant downregulation of AKT, ERK1/2, and Cyclin D1 protein levels after treatment with CLM29 and CLM3 was observed in both biop-pATC and FNA-pATC.

CONCLUSIONI

Our data showed similar sensitivity of pATC obtained by both biopsy and FNA to the TKIs CLM29 and CLM3, as both drugs were able to reduce cell growth and increase apoptosis.
The possibility of testing sensitivity to different TKIs in each patient could open the way to personalized treatments, thus avoiding the administration of ineffective, and potentially dangerous, drugs.

B012

MIMICKING THE "BRCANESS" IN THE HIGH GRADE SEROUS OVARIAN CANCER:ROLE OF PIN1 TARGETING

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INTRODUZIONE

Ovarian cancer (OC) is the fifth leading cause of death among women, especially the High-grade Serous Ovarian Carcinoma (HGSOC) subtype, which is the most common and aggressive, due to its frequent relapse and resistance to chemotherapy.
It's also diagnosed at an advanced stage (III and IV), where the tumour has spread to the abdomen or outside the abdominal cavity, because of the lack of specific symptoms and effective screening strategies. Progresses have been made by identifying BRCA1/2 as high-risk OC-genes. Their loss leads to HR deficiency, genome instability and increased sensitivity to DNA-damaging agents (PT-drugs, PARPis), affecting therapeutic approaches. The "BRCAness" concept was extended also to HDR-related genes deficiency.
One appealing strategy might be the Pin1 isomerase inhibition, as it is able to bind and colocalize with BRCA1 in breast cancer cells, increasing its stability under genotoxic stress. Thus, its depletion reduces the HR process, sensitizing BRCA1-proficient breast cancer cells to PARPis.
Our main aim is to examine the Pin1-BRCA1 relationship in Ovarian Cancer context.

METODI

BRCA1-proficient established HGSOC cell lines were used. We performed: i. Preliminary in silico analysis on protein data collected from HGSOC-bearing patients; ii. in vitro studies of Pin1-BRCA1 interaction under genotoxic stress (Co-immunoprecipitation experiments, lentiviral inducible Pin1 genetic targeting, transient transfections, pharmacological Pin1 inhibition, cell viability assay) and iii. in vivo experiments.

RISULTATI

Pin1-BRCA1 protein levels positively correlates in 39 HGSOC-bearing patients. Furthermore, Pin1 is able to bind BRCA1 under genotoxic stress and its inhibition reduces BRCA1 protein expression, increasing HGSOC cells death and then resulting in their re-sensitization to chemo-therapeutic drugs.

CONCLUSIONI

These findings corroborate our hypothesis of "BRCAness" mimicking via Pin1 inhibition, thus representing a novel therapeutic target useful to improve the clinical efficacy of standard treatments also to BRCA1-proficient OC patients, extending the overall survival of patients regardless their BRCA-mutational status.

B013

DUAL INHIBITION OF SETD8 AND HDAC2 RESTORES P53K382-DEPENDENT CELL DEATH IN PEDIATRIC AND ADULT TUMOR CELLS

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INTRODUZIONE

TP53 (p53) is the most frequently silenced tumor suppressor gene in human cancers by either genetic mutations or functional inactivation. So far, reactivation of WT p53 appears the most pursuable intervention. To this end, a full understanding of the molecular mechanisms of p53 functional inactivation needs to be accomplished. SETD8 is a histone methyltransferase overexpressed in several cancer types including neuroblastoma (NB) and colorectal cancer (CRC), that exerts oncogenic activity via multiple mechanisms. SETD8 is the sole methyltransferase that monomethylates p53 on lysine 382 (p53K382me1), leading to the inhibition of its pro-apoptotic and growth arrest functions. K382 represents a critical site for p53 acetylation, a modification that enhances p53 pro-apoptotic activity, with HDAC2 being a mediator of p53K382 deacetylation.

METODI

To investigate the causal role of p53 activation in response to the SETD8 inhibitors UNC0379, SGSS05-NS3 and to the HDAC2 inhibitor MS275, p53-null CRC and NB cell lines were engineered with inducible vectors to express WT or mutant p53 (p53K382R), which cannot be methylated or acetylated, and subsequently treated with UNC0379, SGSS05-NS3 and MS275 as single agents or in combination. Cell viability and cell-death were assessed by Incucyte Live-Cell imaging and MTT assays.

RISULTATI

Genetic rescue experiments in p53-null CRC and NB cell lines showed that WT p53, but not mutant p53K382R, mediates an increased UNC0379 or SGSS05-NS3-induced cell death and MS275-induced growth arrest accompanied by enhanced mRNA levels of p53 pro-apoptotic and pro-growth arrest target genes such as FAS and BAX or p21 and GADD45, respectively. Combined SETD8 and HDAC2 inhibition significantly reduced cancer cell viability in WT p53, but not in p53K382R mutants, supporting a p53K382-dependent reduced cell viability in NB and CRC models. scRNA-seq data from CRC tissues reveal that SETD8 and HDAC2 are highly expressed in cancer stem cells and tumor associated-macrophages with p53 signaling downregulated, supporting the dual inhibition of both SETD8 and HDAC2.

CONCLUSIONI

Our findings identify p53K382 as a critical epigenetic regulatory hub controlled by SETD8 and HDAC2 and reveal a previously uncharacterized dual mechanism of p53 repression in cancer cells. Overall, these findings pave the way for innovative therapeutic strategies rescuing p53 functions in pediatric and adult tumors.

B014

ANTINEOPLASTIC EFFECTS OF DOXORUBIZEN IN ANAPLASTIC THYROID CANCER: EVALUATION BY IN VITRO AND IN VIVO STUDIES

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INTRODUZIONE

Anaplastic thyroid cancer (ATC) is the most aggressive hystotype of TC whose current treatments allow a median survival of about 6 months, and an overall survival of about 20%. For these reasons new therapies are urgently needed. We aim to investigate a new drug doxorubizen, a dual-action structural hybrid (chimera) of doxorubicin and DNA methylating drug temozolomide.

METODI

We tested doxorubizen and lenvatinib (a tyrosine kinase inhibitors already approved in Japan for the treatment of ATC), alone or in combination, in comparison to doxorubicin.
We performed in vitro studies in continuous 8305C cell lines and in five primary human ATC cell (pATC) cultures and subsequently we carried out in vivo studies in CD nu/nu mice by injecting continuous and pATC, in order to evaluate the antineoplastic effect of doxorubizen and doxorubicin.

RISULTATI

All drugs showed a significant antiproliferative and proapoptotic effect in 8305C and in pATC, in vitro. Noteworthy, doxorubizen demonstrated a higher antiproliferative and proapoptotic effect compared to doxorubicin or lenvatinib, showing also a stronger effect in the inhibition of migration in pATC cells. The dual treatment of doxorubizen plus lenvatinib showed that doxorubizen is capable of acting synergistically in vitro with lenvatinib enhancing its antiproliferative effect, whereas doxorubizen alone is the primary factor promoting the proapoptotic effect.
Moreover, doxorubizen significantly reduced cytokine/chemokine production in ATC cells.
We have performed also in vivo studies that demonstrated a better activity of doxorubizen in tumor suppression and weight reduction compared to doxorubicin.

CONCLUSIONI

Overall, our results demonstrate that doxorubizen exerts significant antineoplastic activity in both the 8305C and pATC cells in in vitro and in vivo experimental models, supporting further evaluation of doxorubizen as a potential therapeutic strategy for patients with ATC.

B015

HUR TARGETING ENHANCES TUMOR IMMUNOGENICITY ACROSS MULTIPLE CANCER MODELS

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INTRODUZIONE

Immune evasion is a hallmark of cancer and is frequently associated with impaired antigen presentation, an immunosuppressive tumor microenvironment, and poor responsiveness to immune checkpoint inhibitors (ICIs). The RNA-binding protein HuR (ELAVL1) is overexpressed in several malignancies and regulates transcripts involved in inflammation, tumor progression, and immune regulation. Although HuR has been implicated in immune evasion through PD-L1 regulation, its role in antigen presentation remains poorly defined. Here, we investigated whether HuR targeting modulates tumor immunogenicity across different cancer models.

METODI

Human EGFR-mutant NSCLC (PC9, H1975), their osimertinib-resistant derivatives (PC9OR, H1975OR), and relative HuR-CRISPR/Cas9-KO, murine melanoma (B16F10, B16F10-OVA) and colorectal cancer (MC38-OVA) cell lines were used. HuR was pharmacologically inhibited with KH-3. RNA-seq (Genomix4Life S.r.l) and GSEA were performed on OR/KO cells following HuR inhibition/deletion. MHC class I expression was assessed by flow cytometry and IL-6 secretion by ELISA.

RISULTATI

Transcriptomic analyses showed that HuR depletion activates the IFNγ response, with upregulation of genes involved in antigen presentation programs. Consistently, similar findings from an independent HuR-silencing dataset of malignant peripheral nerve sheath tumor cell line (ST88-14) support a conserved role for HuR in immune regulation. In EGFR-mutant NSCLC cells, both genetic and pharmacological HuR targeting reduced IL-6 secretion and increased MHC class I levels, with partial reversal by exogenous IL-6, suggesting that pathways beyond the IL-6/STAT3 axis contribute to this effect. Importantly, Notably, HuR inhibition also enhanced H-2Kb/H-2Db expression and OVA peptide presentation in murine melanoma and colorectal cancer models indicating that its immunomodulatory effects extend beyond lung cancer.

CONCLUSIONI

HuR acts as a negative regulator of antigen presentation and immune recognition in cancer cells. Across multiple tumor models, HuR inhibition activates IFNγ-associated programs, enhances MHC class I expression, and reduces immunosuppressive signaling. These findings support HuR targeting as a potential strategy to increase tumor immunogenicity and improve responsiveness to immunotherapy.

B016

LARGE-SCALE METABOLOMIC ASSESSMENT OF OBESITY USING MULTIPLE ANALYTICAL PLATFORMS

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INTRODUZIONE

Obesity is commonly defined using body mass index (BMI), but BMI does not fully reflect differences in adiposity distribution, metabolic status, or cardiometabolic risk. This study aimed to describe the metabolic profile associated with higher BMI in a large multi-cohort setting and to assess whether this profile is consistent across different populations.

METODI

Circulating metabolites from nine cohorts in six countries within the BETTER4U (https://better4u.eu/) consortium were analysed, including 314,767 participants. Three targeted metabolomics platforms were used: Nightingale Health , Biocrates , and Metaboneer. In each cohort, associations between BMI and metabolite concentrations were tested using regression models adjusted for age and sex. The results from the individual cohorts were then pooled using random-effects meta-analysis. This approach was chosen to account for possible differences between cohorts. Meta-regression was also used to investigate whether part of this variability could be explained by geographic region.

RISULTATI

Higher BMI was associated with a consistent metabolic pattern. Positive associations were observed with triglyceride-rich lipoproteins, especially large and very large VLDL particles, serum triglycerides, glycerol, branched-chain and aromatic amino acids, glycoprotein acetyls, and the ApoB/ApoA-I ratio. Negative associations were found for large and very large HDL-related measures, including HDL particle concentration and cholesterol, phospholipid, and total lipid content. Lower levels of glycine, glutamine, choline-related metabolites, phosphatidylcholines, and lysophosphatidylcholines were also observed. Finally, geographic region explained part of the variability observed for some metabolites, suggesting that the metabolic alterations associated with BMI are not identical across populations.

CONCLUSIONI

This large multi-platform metabolomics study identifies a reproducible metabolic signature of higher BMI, mainly characterized by VLDL expansion, HDL remodeling, altered amino acid metabolism, and inflammatory-related changes. These results support the use of metabolomic information, together with BMI and routine clinical markers, to better characterize obesity-related metabolic risk.

B017

ONE MOLECULE, DUAL FUNCTION: A NOVEL SCDB-TRAIL-ICG STRATEGY FOR IMAGING AND TARGETED THERAPY IN TRIPLE-NEGATIVE BREAST CANCER

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INTRODUZIONE

The development of agents combining tumor-selective targeting, effective cancer cell killing, and real-time imaging remains a major challenge in precision oncology, particularly for triple-negative breast cancer (TNBC). TRAIL (Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand) selectively induces apoptosis in cancer cells but has shown limited clinical efficacy due to poor pharmacokinetics and insufficient tumor selectivity. To overcome these limitations, we exploited the hERG1/β1 integrin complex, a tumor-associated target identified by our group and selectively expressed in several solid tumors. Building on our patented single-chain diabody directed against this complex (WO2019/015936; Duranti et al., Mol Cancer Ther. 2021), we developed a multifunctional scDb-TRAIL construct (Duranti et al., Mol Cancer Ther. 2025) with both therapeutic and diagnostic potential.

METODI

A recombinant scDb-TRAIL fusion protein was generated in CHO cells by linking three TRAIL moieties to an scDb targeting the hERG1/β1 integrin complex. Antitumor activity was evaluated in TNBC cell lines, 3D microfluidic tumor-on-chip models, and murine xenografts. For imaging studies, the construct was conjugated with Indocyanine Green (ICG) and assessed for selective tumor detection.

RISULTATI

scDb-TRAIL retained high specificity for hERG1/β1-positive breast cancer cells and induced a marked reduction in tumor viability through apoptosis. Therapeutic efficacy correlated with expression levels of both the hERG1/β1 complex and TRAIL receptors (DR4/DR5), supporting a biomarker-driven mechanism of action. In vivo, treatment significantly inhibited TNBC tumor growth while maintaining a favorable safety profile, with no relevant off-target toxicity. Conjugation with ICG enabled highly selective visualization of tumor lesions, demonstrating the dual therapeutic and diagnostic functionality of the platform.

CONCLUSIONI

Our findings identify scDb-TRAIL-ICG as a promising next-generation theranostic agent for triple-negative breast cancer, integrating tumor-selective imaging with targeted apoptosis induction in a single molecular platform. This strategy represents a highly innovative approach toward precision oncology and supports further translational development for personalized treatment of aggressive solid tumors.

B018

PHOSPHOLIPID REACYLATION ACTS AS A METABOLIC CHECKPOINT OF MACROPHAGE ACTIVATION AND RESISTANCE TO LEISHMANIA INFECTION

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INTRODUZIONE

Leishmaniasis is a parasitic disease with diverse clinical outcomes determined by host immunity and parasite species. Macrophages are the primary host cell for Leishmania, and their activation state critically influences infection outcome, yet its metabolic basis remains poorly understood. Lysophosphatidylcholine acyltransferase 2 (LPCAT2), a phospholipid remodelling enzyme, is enriched in inflammatory macrophages and co-localises with NOS2+ in Leishmania-infected cells in an in vivo murine model, suggesting a role in inflammatory competence.

METODI

Human monocyte-derived macrophages (MDMs) were polarised towards pro-inflammatory (IFNγ+TNFα) or regulatory (IL-4) phenotypes and infected with L. infantum or L. tropica; infection rates and cytokine secretion were assessed. Building on evidence of LPCAT2 enrichment in granuloma macrophages, we investigated LPCAT inhibition in vitro. Bone marrow-derived macrophages (BMDMs) were stimulated with LPS+IFNγ ± TSI-01 and infected with L. donovani; nitric oxide, activation markers and transcriptional changes were analysed by Griess assay, flow cytometry and scRNA-seq. Susceptibility to L. donovani was evaluated by flow cytometry and Giemsa staining.

RISULTATI

Pro-inflammatory MDMs displayed lower L. infantum infection rates than regulatory macrophages at 48h (47.3% vs 66.6%, $p<0.05$), with increased TNFα and reduced IL-10. Interestingly, scRNA-seq of BMDMs identified LPCAT2 expression predominantly in LPS+IFNγ-stimulated clusters; while flow cytometry confirmed enrichment of NOS2 and TNF in LPCAT2-high cells. In BMDMs, LPCAT2 inhibition increased susceptibility to L. donovani infection (80.95% vs 90.85%, $p<0.0001$), with higher parasite burden. LPCAT2 inhibition reduced nitric oxide production and induced transcriptional reprogramming, including upregulation of Cxcl2 and Cxcl3.

CONCLUSIONI

In both models, pro-inflammatory macrophages showed greater Leishmania resistance, suggesting LPCAT2-mediated remodelling as a checkpoint of macrophage inflammatory activation. Its inhibition shifts transcriptional state and increases parasite susceptibility. This will be validated in human macrophages using pharmacological LPCAT2 inhibition, to assess therapeutic relevance in leishmaniasis.

B019

DNA METHYLATION REMODELLING IN WHOLE BLOOD FROM RHEUMATOID ARTHRITIS PATIENTS TREATED WITH IMMUNE-MODULATORY METHOTREXATE THERAPY

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INTRODUZIONE

Rheumatoid arthritis (RA) is an autoimmune disorder marked by synovial inflammation and systemic immune dysregulation. Although methotrexate (MTX) induces remission in consistent percentage of patients, its effect on DNA methylation (DNAm) of circulating immune cells is unknown and is worth to be investigated owing to its possible role on the short and long term effect of MTX therapy.

METODI

We performed a longitudinal genome-wide DNAm analysis in RA patients (N=15) at baseline and after MTX-induced clinical remission. Peripheral blood DNA was profiled using Illumina EPIC arrays. Analyses included differential methylation (DMPs/DMRs), stochastic epigenetic mutations (SEMs), and DNAm-based inflammatory scores. Significant findings were compared with healthy controls (HC) from an external cohort.

RISULTATI

MTX treatment induced significant, specific epigenetic remodelling signatures. Notably, while MTX did not affect epigenetic ageing across available algorithms, a significant increase in SEM burden was observed at follow-up, along with a more stable SEM landscape in RA patients compared with HC, suggesting altered epigenetic variability dynamics. Epivariation analysis showed enrichment of inflammation-associated loci, including DUSP22, previously linked to RA activity and severity.

Longitudinal analysis identified few differentially methylated CpGs, including a locus in CDC42EP5 gene that shifted toward healthy control levels after treatment. This gene interacts with CDC42, a small Rho GTPase altered in RA.

DNAm-CRP scores strongly correlated with serum CRP at baseline but showed reduced correlation after treatment, suggesting a partial decoupling between systemic inflammation and its epigenetic signature.

CONCLUSIONI

MTX treatment is associated with specific DNAm signatures that are functionally linked to RA pathophysiology, immune modulation, and inflammation. Despite the relatively small sample size, the analysis of this well-characterized patient's cohort supports the hypothesis that DNAm could play a role in the MTX response in RA and should be useful in patient's monitoring and advanced prognostic applications.

B020

TARGETING THE NF-κB/CES1 AXIS TO REDUCE CANCER STEM CELL SURVIVAL IN NSCLC

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INTRODUZIONE

Non-small cell lung cancer (NSCLC) is one of the most prevalent and therapy-resistant malignancies worldwide. One of the main challenges in therapy is treatment resistance and tumor relapse, often associated with the presence of cancer stem cells (CSCs), a small tumor subpopulation (0,001-2%) regulated by multiple signaling pathways. Among these, the NF-κB pathway plays a key role in CSC stemness and metabolic regulation. However, direct NF-κB inhibitions is associated with significant side effects. Therefore, targeting downstream metabolic effectors, such as carboxylesterase 1 (CES1), may represent a promising alternative therapeutic strategy to overcome tumor resistance.

METODI

CSCs were generated from three NSCLC cell lines (A549, CALU-3, and H2228) through a specific dedifferentiation protocol. Acquisition of the CSCs phenotype was validated by morphological analysis and by the expression of established stemness markers using microscopy and qRT-PCR, respectively. Seahorse XF-96 Analyzer, qRT-PCR and Cell-Titer Glo viability assays were performed to assess metabolic profiling, CES1 expression and the therapeutic efficacy of CES1 inhibitor.

RISULTATI

Metabolic profiling showed that CSCs exhibit a more quiescent state compared to parental cells. CSCs also showed increased expression of CES1 relative to parental cells. Under basal condition, the treatment of CSCs with the CES1 inhibitor GR148672X or cisplatin did not result in significant differences in cell viability between treatments. However, under metabolic stress conditions, CES1 inhibition diminished CSCs cell survival exhibiting a lower IC50 value compared to cisplatin, suggesting a potential role of CES1 in metabolic adaptation of these cells.

CONCLUSIONI

These results suggest that CES1 may play a role in the metabolic plasticity of CSCs, particularly under stress conditions. Although further studies are needed to validate CES1 as a potential therapeutic target in CSCs, targeting metabolic downstream effectors of the NF-κB pathway may represent a promising strategy to overcome therapy resistance in NSCLC.

B021

POLD1 VARIANTS IN A COHORT OF HEREDITARY CANCER PATIENTS

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INTRODUZIONE

Biallelic POLD1 alterations have been associated with severe multisystem developmental disorders (immunodeficiency, growth impairment, neurodevelopmental abnormalities). In contrast, heterozygous pathogenic variants affecting the exonuclease domain of POLD1, although rare (<1%), are associated with hereditary cancer syndromes, mainly including colorectal cancer (CRC), endometrial cancer (EC) and the Polymerase Proofreading-associated polyposis (PPAP).

METODI

We analyzed the prevalence and clinical phenotype of POLD1 variants in 652 patients with suspected hereditary cancer syndromes, who underwent genetic counseling and germline multigene panel testing. Variants of uncertain significance (VUSs) and a novel variant were evaluated using bioinformatic tools. Furthermore, mRNA analysis from one patient blood sample, was performed for a novel intronic variant identified in our laboratory.

RISULTATI

POLD1 variants were identified in 0.76% (5/652) of patients. Three distinct variants were detected: two exonuclease-domain VUSs, c.887T>G (p.Val296Gly) and c.965G>A (p.Arg322His), and one novel intronic variant, c.971-78G>T, identified in three patients. Genotype–phenotype analysis revealed that the three female patients carrying the novel variant presented with: (i) Breast Cancer (BC) at 45 years of age and family history of BC (ii) CRC at 18 years of age and family history of BC, EC and melanoma; (iii) BC at 65 years of age and family history of BC. The two patients carrying the VUSs were both women, the first one with BC diagnosed at 48 and the second one with BC at 63 years of age and EC at 65 years of age. Bioinformatic analysis classified both the missense variants as VUSs (class III), whereas c.971-78G>T was classified as likely benign (class IV). mRNA analysis demonstrated that c.971-78G>T does not alter splicing.

CONCLUSIONI

Our findings confirm the rarity of POLD1 variants in hereditary cancer syndromes. The lack of splicing alterations, together with bioinformatic classification, mRNA analysis, and recurrence in three patients, supports the benign nature of the c.971-78G>T variant, demonstrating that it is not responsible for the cancer phenotype observed in carriers and affected relatives. Further studies are needed to clarify the role of VUSs.

B022

DUAL TARGETING OF CAIX AND FGF/FGFR SYSTEM IN TRIPLE NEGATIVE BREAST CANCER

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INTRODUZIONE

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, characterized by the lack of targeted therapies, frequent development of drug resistance, and high recurrence rates. Standard neoadjuvant chemotherapy often fails to achieve pathological complete response in TNBC, highlighting the need for novel therapeutic targets. Among the proteins involved in hypoxia-dependent response, carbonic anhydrase IX (CAIX) has been reported to correlate with cell proliferation, migration and invasion in several cancer types, including TNBC, where CAIX is expressed also in normoxic condition. Similarly, Fibroblast growth factor (FGF)/FGF receptor (FGFR)-mediated signaling sustains tumor progression, and has been reported to be aberrantly activated in TNBC.
Aim of this project is to investigate the therapeutic potential of dual CAIX and FGFR inhibition in TNBC using clinical-grade drugs.

METODI

Here, CAIX, FGF and FGFR expression was assessed in human TNBC cell lines in both normoxic and hypoxic conditions by qPCR and western blot analysis. TNBC cells were treated with the CAIX inhibitor SLC-0111 and the pan-FGFR inhibitor erdafitinib, either alone or in combination, to evaluate the effects of single or dual CAIX/FGFR inhibition on malignant features, including proliferation, migration, and clonogenic growth.

RISULTATI

We observed that the FGF/FGFR signaling network is broadly active in TNBC cells, and that CAIX is expressed in normoxia and is upregulated under hypoxic conditions. Consistent with a pro-tumor role exerted by CAIX and the FGF/FGFR system in TNBC progression, treatment with SLC-0111 and erdafitinib significantly reduced TNBC cell proliferation, migration, and clonogenic potential, with the combination treatment showing an additive effect.

CONCLUSIONI

Collectively, these preliminary data support the therapeutic potential of the dual inhibition of CAIX and FGFR in reducing malignant properties of TNBC.

B023

METABOLIC AND IMMUNE SIGNATURES ASSOCIATED WITH REJECTION AND TOLERANCE IN LUNG TRANSPLANTATION

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INTRODUZIONE

Lung transplantation is a surgical procedure designed to replace one or both diseased lungs with healthy donor organs in patients with end-stage pulmonary diseases.
After transplantation, chronic rejection, clinically defined as chronic lung allograft dysfunction, remains the leading cause of graft failure, with a 5-year survival rate of 55%.
A deeper knowledge of the immunological scenarios associated with graft tolerance or rejection could enable the selective targeting of alloreactive T cells.

METODI

We collected transbronchial biopsies from transplanted patients and performed gene expression analysis by RT-PCR, focusing on immunometabolic pathways, including MYC and Programmed Cell Death Protein-1 (PD-1) related pathways. In parallel, we collected peripheral blood samples from patients; we quantified circulating cell-free mtDNA in plasma using Droplet Digital PCR and we isolated CD8+ T cells from the cellular fraction and assessed their metabolic profile at baseline and after stimulation with antiCD3/antiCD28 antibodies, to mimic T cell activation.

RISULTATI

Spearman's rank correlation revealed a strong correlation among the selected immunometabolic genes. We observed higher PD-1 expression in tissues from patients experiencing rejection. CD8+ T cells from patients without rejection showed higher basal PD-1 expression with only a modest increase after stimulation, consistent with a partially exhausted or regulated T cell state. In contrast, rejecting patients displayed higher baseline MYC levels, reflecting active effector and metabolic responses. Subjects experiencing rejection also showed higher levels of cell free mtDNA in plasma.

CONCLUSIONI

Gene expression analysis in transbronchial biopsies suggests that these factors might be part of the same regulatory network involved in metabolic pathways after transplantation, and that PD-1 could be an important biomarker of acute rejection. Elevated circulating cell-free mtDNA in rejecting patients may reflect tissue damage and systemic inflammation, while findings in peripheral CD8+ T cells indicate that targeting metabolic pathways associated with effector function, while supporting exhaustion-related programs, could help modulate alloreactive responses and enhance graft tolerance.

B024

PHARMACOLOGICAL AND GENETIC TARGETING OF HUR IMPAIRS ONCOGENIC TRANSCRIPTION IN TKI-RESISTANT NSCLC

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INTRODUZIONE

Human Antigen R (HuR) is an RNA-binding protein frequently overexpressed or mislocalized in cancer, including lung adenocarcinoma. HuR regulates RNA splicing in the nucleus and mRNA stability/translation in the cytoplasm. Our preliminary data showed increased HuR expression in EGFR-mutant NSCLC cells following resistance to EGFR-tyrosine kinase inhibitors (TKIs), identifying HuR as a novel determinant of EGFR-independent TKI resistance whose pharmacological inhibition reduced cell proliferation by impairing cell cycle progression. Here we investigated the molecular mechanisms underlying HuR targeting in EGFR-TKI-resistant settings.

METODI

EGFR-TKI-resistant cell lines (PC9OR/H1975OR) were generated by treating PC9/H1975 cells with increasing Osimertinib concentrations. Pharmacological HuR inhibition was achieved using SRI-42127, KH3 and TM7 (kindly provided by A. Provenzani). PC9 HuR knockout (KO) cells served as a genetic loss-of-function model. Protein expression was analyzed by immunofluorescence and immunoblotting. RNA-seq (Genomix4Life S.r.l) and GSEA were performed on OR and KO cell lines following HuR inhibition/deletion.

RISULTATI

PCA revealed inhibitor-dependent transcriptional heterogeneity, with SRI-42127-treated OR cell lines clustering together, whereas KH3 and TM7 induced distinct profiles. Hierarchical clustering identified five transcriptional modules, including a proteostasis-related cluster enriched in ribosome biogenesis, RNA metabolism, protein folding, and cell-cycle functions, representing a major HuR-dependent vulnerability. GSEA highlighted both inhibitor-specific and shared pathway alterations, including suppression of cell-cycle progression (G2M checkpoint, mitotic spindle assembly), MYC, and E2F signatures, consistent with the antiproliferative effects of HuR inhibition. Similar transcriptional changes in PC9 HuR KO cells confirmed on-target activity.

CONCLUSIONI

Collectively, our results identify HuR as a critical regulator of the transcriptional landscape of EGFR-TKI-resistant NSCLC and provide a rationale for its therapeutic targeting. The shared and inhibitor-specific transcriptional effects observed warrant further investigation to define the pharmacodynamic and mechanistic properties of currently available HuR inhibitors.

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B025

EVALUATION OF THE MICRORNA EXPRESSION PROFILE SUGGESTS THE INVOLVEMENT OF THE HIPPO PATHWAY IN COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy worldwide (Tu, 2022). Unraveling novel molecular pathways underlying CRC development is essential to improve disease diagnosis, prognosis, and treatment. The present study aimed to evaluate the global microRNA expression profile in tumor tissue and paired adjacent healthy mucosa from CRC patients, to identify the molecular pathways deregulated by microRNA aberrant expression.

METODI

Samples were collected from the Department of Surgery, University of Cagliari (Italy). RNA samples were extracted from tumor tissue and paired healthy mucosa from 40 CRC patients. Samples from 6 patients was analyzed using a microRNA Assay (Illumina iScan System), enabling the simultaneous profiling of approximately 700 microRNAs. MicroRNAs and proteins of interest were subsequently validated in the entire cohort by quantitative Real-Time PCR (qRT-PCR) and Western blot analysis, respectively. Statistical analysis was performed using Prism software (Version 7).

RISULTATI

Microarray analysis revealed approximately 28 deregulated microRNAs in tumor tissue compared with paired healthy tissue in at least 5 out of 6 patients. Among these, miR-125b, miR-133a, miR-183, miR-30a, and miR-31 emerged from bioinformatic analysis as key regulators of the Hippo signaling pathway, suggesting its involvement in CRC development and progression. qRT-PCR validation demonstrated that miR-125b was downregulated in 80% of tumor samples within the 40-patients cohort. Furthermore, Western blot analysis revealed a significant upregulation of YAP, a major Hippo pathway effector, in tumor vs normal tissue ($p<0.0001$). The expression of Survivin, a YAP target gene, was subsequently analyzed in 24 CRC patients previously evaluateed for YAP expression. Survivin was also significantly overexpressed in tumor tissue compared to paired normal tissue ($p=0.0018$).

CONCLUSIONI

The combined analysis of deregulated microRNA and the increased expression of Hippo pathway effectors, YAP, and Survivin, suggest the involvement of the Hippo pathway in CRC. Given the established role of the Hippo pathways in tissue repair, proliferation, apoptosis and cell regeneration (Moroishi, 2015; Pibiri and Simbula, 2022), these findings further support its potential contribution in tumor development and progression.

B026

ACCELERATED EPIGENETIC AGING AND EPIGENOMIC SIGNATURES IN MAJOR DEPRESSIVE DISORDER: PRELIMINARY RESULTS FROM THE OPADE PROJECT

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INTRODUZIONE

Major Depressive Disorder (MDD) is among the most prevalent and disabling psychiatric conditions worldwide, affecting approximately 280 million individuals. Despite the availability of several pharmacological treatments, clinical outcomes remain suboptimal, with a considerable proportion of patients exhibiting limited or no response to therapy. This inter-individual variability underscores the unmet need for objective molecular biomarkers capable of predicting treatment response and supporting personalized therapeutic strategies. The OPADE project (EU Horizon Europe, GA No. 101095436) is an international multicenter initiative involving 14 research and clinical centers across 10 countries, aiming to integrate multi-omics data to identify clinically relevant molecular signatures in MDD. Within this framework, CEINGE is responsible for genomic and epigenomic profiling of patients longitudinally monitored throughout pharmacological treatment.

METODI

Genomic DNA was extracted from blood and saliva samples and underwent genome-wide methylation analysis using the Epic Array Illumina platform. Bioinformatic approaches were used to identify depression-related epigenomic signatures, differentially methylated CpG sites, and measures of epigenetic age acceleration.

RISULTATI

Genome-wide DNA methylation analyses were performed on a preliminary cohort of approximately 100 patients assessed at baseline and after one year of treatment. Early findings indicate a statistically significant increase in epigenetic age compared to chronological age across samples, suggesting a pattern of accelerated epigenetic aging in patients with MDD. Furthermore, longitudinal comparisons between baseline and follow-up samples reveal more than 100,000 differentially methylated sites, indicating extensive and dynamic epigenomic remodeling associated with disease progression, clinical improvement, and/or treatment exposure.

CONCLUSIONI

These preliminary findings support the hypothesis that MDD is associated with widespread epigenomic alterations and a measurable acceleration of epigenetic aging. The large number of differentially methylated sites observed over time highlights the dynamic nature of the epigenome in this clinical context. Integration of these data with metabolomic, proteomic, and microbiome profiles within the OPADE consortium will facilitate the identification of robust molecular signatures of disease and treatment response, ultimately supporting the development of precision medicine approaches in psychiatry.

B027

EVALUATING PLASMA EXTRACELLULAR VESICLE GALECTIN-10 AS A NON-INVASIVE BIOMARKER FOR EOSINOPHILIC ESOPHAGITIS: A PILOT STUDY

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INTRODUZIONE

Eosinophilic Esophagitis (EoE) is a chronic, Th2/eosinophilic disease driven by genetic, environmental, and epithelial barrier factors. Currently, invasive biopsies to gain eosinophil (Eos) counts in esophageal mucosa (EM) are required for EoE diagnosis and monitoring, highlighting an unmet need for non-invasive biomarkers. Previous studies identified in the EM of EoE patients a subset of CD16+ Eos with impaired immunosuppressive function, partially mediated by the Eos-specific protein Galectin-10 (Gal-10), which is also released in the EM via extracellular vesicles (EVs). Both EM CD16+ Eos and Gal-10+ EVs correlated with response to therapy. This pilot study aims to identify Gal-10+ EVs in peripheral blood samples from EoE patients and non-EoE disease controls.

METODI

Plasma EVs were isolated from study subjects after informed consent for blood drawing was obtained. Plasma samples were processed by differential ultracentrifugation for EV isolation. Nanoparticle Tracking Analysis (NTA) characterized EV concentration and size distribution. Western blot (WB) assessed the specific EV markers CD9 and CD63, and Gal-10 expression.

RISULTATI

Along with clinical and EM Eos count, plasma EVs were isolated from EoE (n=4), eosinophilic non-GI diseases [atopic dermatitis (AD, n=2), hypereosinophilia (HE, n=1)], non-eosinophilic GI disease [achalasia (AC, n=3)], and healthy controls (HC) (n=2). EoE samples were evaluated pre- (T0) and post- (T1) treatment (food elimination diet, n=3; topical budesonide, n=1). Clinical response (R) or non-response (NR) (n=2 each) was defined by T1 EM Eos count. NTA identified EV particles with a mode diameter (76.6–145.3 nm) compatible with exosomes. Plasma EV protein concentrations ranged from 27 to 165 µg/mL. WB confirmed CD9 and CD63 expression. Gal-10 was undetectable in HC and AC, but clearly expressed in eosinophilic conditions (AD, HE) and in 3 out of 4 EoE patients at T0. In T1, EoE NR patients Gal-10 remained elevated while in one R patient EVs concentration and Gal-10 levels
were reduced.

CONCLUSIONI

This pilot study reports the detection of Gal-10+ EVs in the plasma of EoE patients. If validated in larger cohorts, Gal-10+EVs may reflect high EM Eos and may be evaluated as a non-invasive marker for EoE diagnosis and/or follow-up.

B028

AN INFLAMMASOME-ASSOCIATED MOLECULAR AND TISSUE SIGNATURE CHARACTERISES TEMPORAL ARTERY INFLAMMATION IN GIANT CELL ARTERITIS

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INTRODUZIONE

Giant cell arteritis (GCA) is a granulomatous large-vessel vasculitis associated with ischemic complications. Although adaptive immune responses have been extensively investigated in GCA, increasing evidence suggests that innate immune pathways may contribute to vascular inflammation. We investigated the expression of inflammasome-related molecules and cytokines in temporal artery biopsies from patients with GCA.

METODI

Temporal artery biopsies (TABs) from treatment-naïve patients with GCA showing transmural inflammation (TMI) or non-transmural inflammatory changes, together with non-vasculitic arterial controls, were collected at the Rheumatology Unit of Ospedale Santa Maria Nuova, Reggio Emilia, and the University of Campania "Luigi Vanvitelli", Naples. Immunohistochemistry was performed on FFPE sections to assess IL-1β, NLRP3, AIM2, IL-32 and IL-33 expression. Gene expression was assessed by RNA sequencing on frozen TABs, NanoString analysis on FFPE TABs, and RT-PCR. Positivity rates and fold changes for AIM2, NLRC4, NLRP3, IL18, IL32 and IL1B were compared between inflamed and non-inflamed TABs.

RISULTATI

RNA sequencing of frozen TAB samples showed inflammasome-related gene upregulation, including AIM2, IL18, IL1B, IL32, NLRC4, and NLRP3, in the TMI group compared with negative controls, with fold changes of approximately 3–4 and significant p-values. NanoString analysis of FFPE TABs confirmed significant upregulation of inflammasome-related genes, including IL18, IL1B, IL32 and NLRP3, in TMI samples compared with negative TABs, with fold changes ranging from approximately 4.2 to 6.6. Comparison of RNA sequencing and NanoString data revealed a concordant upregulation of inflammasome genes across platforms, supporting the robustness of this molecular signature. RT-PCR confirmed increased expression of AIM2 and NLRP3 in inflamed TABs compared with normal TABs, with higher AIM2 and NLRP3 positivity rates in inflamed samples. In contrast, NLRC4 showed a modest, non-significant increase. Immunohistochemical analysis demonstrated increased vascular expression of IL-1β, NLRP3, AIM2, IL-32 and IL-33 in TABs from patients with GCA, whereas non-vasculitic control arteries showed no detectable staining for these markers.

CONCLUSIONI

By integrating transcriptomic and tissue-level analyses, our study provides evidence of inflammasome pathway activation within the inflamed arterial wall of patients with GCA, supporting a role for innate immunity in GCA pathogenesis.

B029

RED CELL DISTRIBUTION WIDTH IN SYSTEMIC SCLEROSIS: FROM A HEMATOLOGICAL PARAMETER TO A POTENTIAL SYSTEMIC BIOMARKER

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INTRODUZIONE

Red cell distribution width (RDW) is a routine hematological parameter that quantifies erythrocyte anisocytosis. It has recently emerged as a simple, low-cost and widely available biomarker associated with chronic inflammation and oxidative stress in several pathological conditions. In systemic sclerosis (SSc), a disease characterized by diffuse microvascular damage and progressive organ fibrosis, RDW may reflect vasculopathy and clinical severity.

METODI

We conducted a single-center retrospective study on 70 patients with SSc followed at the Rheumatology Unit of the University of Campania "Luigi Vanvitelli". Demographic and serological data, microvascular and cutaneous features (capillaroscopy, digital ulcers, telangiectasias, mRSS), cardiac parameters (systolic/diastolic function, LVEF, pericardial effusion), respiratory function (FVC, TLC, DLCO, ILD) and laboratory indices (Hb, ESR, CRP) were collected. Patients were stratified according to RDW-SD values (normal vs increased), and associations with vascular, fibrotic and cardiopulmonary involvement were evaluated.

RISULTATI

Compared to patients with normal RDW-SD, those with increased RDW-SD more frequently exhibited an active/late capillaroscopic pattern (80.6% vs 35.3%; p=0.002), a greater prevalence of telangiectasias and digital ulcers, and higher CRP levels despite comparable hemoglobin levels, indicating more advanced microvascular damage. They also more often had diffuse cutaneous SSc (dcSSc) and higher mRSS, consistent with a greater fibrotic burden. Elevated RDW-SD was associated with raised PAPs (57.1% vs 22.2%; p=0.013), more frequent diastolic dysfunction, pericardial effusion and increased LVEF. Regarding pulmonary involvement, patients with increased RDW-SD showed a higher prevalence of ILD (64.4% vs 35.0%; p=0.028), reduced TLC% (19.2% vs 2.0%; p=0.018) and a higher percentage of subjects with DLCO <60% (48.7% vs 6.3%; p=0.003). Overall, elevated RDW-SD tracked with a more pronounced vasculopathic–fibrotic phenotype and more extensive organ damage.

CONCLUSIONI

Increased RDW-SD was associated with a more aggressive clinical profile and greater organ involvement in SSc, supporting its potential value as an accessible biomarker for risk stratification and disease monitoring.

B030

APPLICATION OF EMIL (EPIGENETIC MARKER HYPOMETHYLATED FOR ACUTE PROMYELOCYTIC LEUKEMIA) FOR EPIGENETIC PROFILING AND PROGNOSTIC ASSESSMENT IN NON-APL HEMATOLOGICAL MALIGNANCIES

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INTRODUZIONE

Epigenetic dysregulation is a well-established pathogenetic hallmark of hematological malignancies. EMIL (Epigenetic Marker Hypomethylated for Acute Promyelocytic Leukemia, APL) was developed based on the epigenetic DNA hypomethylation signature of APL, linked to poor outcomes (patent EP3458607). EMIL is implemented as an in vitro diagnostic assay based on methylated DNA immunoprecipitation coupled with real-time PCR, for rapid and cost-effective assessment of locus-specific methylation profiles. In this study, we explored whether EMIL-derived methylation profiles could also provide clinically relevant prognostic information in non-APL hematological malignancies.

METODI

A total of 20 hematological patients (median age, 57 years; M/F 10/10) followed at the Hematology and Transplant Center of Salerno were retrospectively included in this study. Genome-wide methylation patterns of selected chromosomal regions were evaluated using the EMIL kit according to the manufacturer's instructions.

RISULTATI

Among the analyzed regions, chromosome 1 methylation status showed the strongest potential prognostic relevance, as patients with low-methylation profiles had a median overall survival (OS) of 51 months. For chromosome 19, the absence of somatic mutations affecting epigenetic regulators appeared to correlate with a low-methylation profile ($p = 0.0703$). In addition, a high-methylation profile involving chromosome 1 was associated with a lower probability of relapse ($p = 0.0792$), whereas high methylation of chromosome 20 correlated with an increased relapse risk ($p = 0.0407$). High-methylation patterns of chromosomes 1 and 20 were significantly associated with each other ($p = 0.0281$), supporting the hypothesis that coordinated epigenetic alterations involving these chromosomal regions may contribute to disease pathogenesis.

CONCLUSIONI

Rapid assessment of methylation status could improve patient risk stratification and support prognostic evaluation of AML patients. Further validation in larger cohorts is required to confirm these preliminary findings. The intellectual property rights related to the EMIL kit (patent EP3458607) are owned by Epi C Srl. The authors, Franci, Altucci, and Dell'Aversana, as shareholders of Epi C Srl, declare a potential conflict of interest. Scala P. is supported by Italian National Recovery and Resilience Plan-PNRR-POC-2023-12377396 CUP Master G13C24000100001—CUP I53C24000560001.

B031

FROM STEM CELL MODELS TO TRNA-BASED THERAPY EXPLORATION FOR IRF2BPL DISEASE

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INTRODUZIONE

NEDAMSS is a severe neurodevelopmental disorder caused by truncating mutations in the intronless IRF2BPL gene. Because IRF2BPL transcripts escape nonsense-mediated decay, mutant cells accumulate truncated proteins that mislocalize to the cytoplasm and form aberrant condensates. Increasing evidence suggests that these aggregates exert a dominant-negative toxic effect through sequestration of the wild-type protein and dysregulation of key developmental pathways, including WNT signaling. This study aims to define the developmental mechanisms underlying NEDAMSS and to evaluate suppressor tRNA (sup-tRNA) technology as a precision therapeutic strategy to restore full-length IRF2BPL protein synthesis.

METODI

To this end, we generated human iPSC lines carrying IRF2BPL pathogenic variants using CRISPR/Cas9 genome editing and established patient-derived iPSC lines through reprogramming of peripheral blood mononuclear cells (PBMCs), followed by neural differentiation.

RISULTATI

Quantitative real-time PCR analysis demonstrated that mutant iPSCs exhibited premature neural commitment associated with altered expression of neural markers and dysregulation of WNT signaling, highlighting the central role of the IRF2BPL/WNT axis in NEDAMSS pathogenesis. Preliminary analyses further showed that pathogenic variants induced aberrant condensates that increased in size and frequency compared with controls, supporting a toxic gain-of-function mechanism. Importantly, sup-tRNA screening demonstrated more than 50% rescue of protein translation for selected constructs, outperforming small-molecule readthrough compounds. In inducible neuronal models, sup-tRNA restored full-length IRF2BPL expression and correct nuclear localization.

CONCLUSIONI

Overall, this study addresses the urgent need for disease-modifying therapies in NEDAMSS by establishing patient-derived iPSC and neural differentiation models to investigate the molecular and developmental mechanisms underlying IRF2BPL-related pathology. Moreover, our pioneering sup-tRNA therapeutic strategy represents a promising personalized approach to correct the genetic defect at its source, with the potential to restore physiological IRF2BPL function and ultimately slow or halt disease progression.

B032

CIRCULATING MICRORNA PROFILES FOR PERSONALIZED MEDICINE IN METASTATIC COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) remains the second leading cause of cancer-related mortality worldwide. Patients diagnosed with stage IV disease face a poor prognosis, with a five-year survival rate of approximately 14%. Liquid biopsy can enable therapeutic personalization, monitor disease evolution and predict treatment response in real-time for individual patients.

METODI

The study involved a discovery cohort of 43 patients with metastatic CRC, sampled at baseline/diagnosis (T0) and during longitudinal follow-up (T1). Notably, the patient population underwent a heterogeneous range of therapeutic regimens rather than a single standardized treatment protocol, reflecting real-world clinical complexity. To manage this variability, a comprehensive data infrastructure was established by integrating clinical, anatomopathological, and molecular imaging data. Circulating microRNAs (miRNAs) were extracted from plasma, microRNA profiles were obtained and bioinformatic analyses were performed.

RISULTATI

Quality control analysis identified 182 miRNAs expressed in all samples. These preliminary findings are based on the longitudinal analysis of a subset of 6 patients at both T0 and T1. Differential expression analysis between Responders and Non-Responders revealed distinct molecular signatures. Of note, at baseline (T0), non-responders exhibited significant upregulation of two miRNAs. Interestingly, one miRNA was consistently downregulated in non-responders across both time-points, while another miRNA emerged as significantly downregulated at T1 specifically in non-responding subjects.

CONCLUSIONI

Our results identify specific miRNA signatures associated with treatment resistance, providing essential biomarkers for precision medicine. Future phases will focus on expanding the cohort and validating these results in independent providing an additional tool for patient management.

B033

EXPLORING THE ROLE OF M6A RNA METHYLATION IN MODULATING BCR-ABL1 EXPRESSION AND ITS FUNCTIONAL IMPACT ON CML AND ALL

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INTRODUZIONE

Chronic Myeloid Leukemia (CML) and Acute Lymphoblastic Leukemia (ALL) are hematologic malignancies that share the BCR-ABL1 fusion protein as a key oncogenic driver. Although tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1 have markedly improved survival in CML, drug resistance and disease relapse remain major clinical challenges. To address these limitations, regulators of the N6-methyladenosine (m6A) RNA modification have emerged as promising therapeutic targets. m6A deposition is orchestrated by a multiprotein “writer” complex composed of METTL3, METTL14, WTAP, VIRMA, and ZC3H13, opposed by “erasers” and interpreted by “readers.” METTL3 serves as the principal catalytic enzyme, while ZC3H13 contributes to the structural stability of the complex. In this study, we investigated the functional interplay between BCR-ABL1 and m6A regulation in both CML and ALL to identify novel therapeutic opportunities.

METODI

Silencing of ZC3H13 resulted in hypomethylation of the BCR-ABL1 fusion transcript. RNA-sequencing analysis revealed a concomitant downregulation of BCR-ABL1 expression, which was further validated at the protein level by Western blot. Additionally, treatment with STC-15, a small-molecule inhibitor that blocks METTL3 enzymatic activity, led to a marked reduction of BCR-ABL1 protein levels across multiple leukemic cell lines.

RISULTATI

Silencing of ZC3H13 resulted in hypomethylation of the BCR-ABL1 fusion transcript. Notably, RNA-sequencing analysis revealed a concomitant downregulation of BCR-ABL1 expression, which was further validated at the protein level by Western blot. Additionally, treatment with STC-15, a small-molecule inhibitor that blocks METTL3 enzymatic activity, led to a marked reduction of BCR-ABL1 protein levels across multiple leukemic cell lines. Compared to ZC3H13, METTL3 inhibition show a very low effect on BCR-ABL1 expression.

CONCLUSIONI

Collectively, these preliminary findings provide new insights into the mechanistic link between m6A RNA modification and BCR-ABL1 expression in leukemia, supporting further investigation of m6A perturbation as potential therapeutic strategies, particularly for patients with TKI-resistant disease.

Keywords: CML; ALL; BCR-ABL1; m6A RNA modification; METTL3; ZC3H13

B034

IDENTIFICATION OF REGULATORY MICRORNA MIR-765 AND MIR-498 FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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INTRODUZIONE

Alzheimer's disease (AD) is characterized by amyloid-beta accumulation, tau hyperphosphorylation, and chronic neuroinflammation. Current therapies offer only symptomatic relief. MicroRNAs (miRNAs) are pivotal post-transcriptional regulators often dysregulated in early AD, making them promising therapeutic targets.
This study aims to characterize the regulatory roles of miR-765 and miR-498 in AD pathogenesis and evaluate their therapeutic potential for mitigating neurodegeneration.

METODI

Bioinformatics tools (TargetScan, miRDB) predicted miR-765 and miR-498 targets. Pathway analysis used GO and KEGG databases. Experimental validation included: (1) miRNA mimic transfection into SH-SY5Y cells, (2) dual-luciferase reporter assays for BAX and PTPRC 3'UTR targeting, (3) RT-qPCR for gene expression, (4) Western blot for protein levels, (5) MTT assays for viability, and (6) Annexin V/PI flow cytometry for apoptosis.

RISULTATI

Preliminary data indicate that both miR-765 and miR-498 are downregulated in AD, with miR-765 showing a 2.3-fold decrease and miR-498 showing a 1.9-fold decrease ($p < 0.01$). Target analysis identified two key regulatory pathways: miR-765 targets BAX in the p53/apoptosis pathway, which controls neuronal cell death, while miR-498 targets PTPRC in the TNF/NF-κB/inflammation pathway, which regulates microglial activation.
Preliminary luciferase assays confirmed direct targeting, with miR-765 reducing BAX luciferase activity by 62% and miR-498 reducing PTPRC luciferase activity by 54% ($p < 0.001$). Preliminary data from miR-765 mimics showed reduced BAX mRNA and protein by 48–51%, decreased apoptosis from 34% to 18% ($p < 0.01$), and improved cell viability by 28%. miR-498 mimics lowered PTPRC expression by 44–47% and reduced inflammatory markers including IL-1β and TNF-α. Combined treatment with both miRNA mimics demonstrated synergistic neuroprotective effects, increasing overall cell viability by 36% compared to Aβ-treated controls.

CONCLUSIONI

miR-765 and miR-498 are downregulated in AD and regulate critical disease pathways: apoptosis through BAX and neuroinflammation through PTPRC. Restoring their levels via mimics reduces neuronal death and inflammation, demonstrating dual neuroprotective effects. These miRNAs represent promising candidates for miRNA-based therapeutics targeting multiple AD mechanisms simultaneously

B035

IDENTIFICATION OF A GUT-DERIVED INFLAMMATORY PHENOTYPE IN END-STAGE KNEE OSTEOARTHRITIS

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INTRODUZIONE

Osteoarthritis (OA) is increasingly recognized as a whole-joint disease characterized by chronic low-grade inflammation. Gut microbiota dysbiosis has emerged as a potential contributor to OA pathophysiology through the gut–joint axis, although its relationship with intestinal and systemic inflammation remains unclear. This study aimed to identify microbiota-associated inflammatory signatures in end-stage knee OA.

METODI

Thirty patients with Kellgren–Lawrence grade 4 knee OA undergoing total knee arthroplasty were enrolled. Plasma interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) were measured by immunoassays. Fecal calprotectin (FC) was quantified as a marker of intestinal inflammation. Gut microbiota composition was assessed by 16S rRNA gene sequencing. Clinical status was evaluated using the Knee Injury and Osteoarthritis Outcome Score (KOOS). Associations were assessed using Spearman's correlation analysis.

RISULTATI

FC concentrations positively correlated with IL-6 (ρ=0.51, p=0.006). IL-6 levels were inversely associated with KOOS Pain (ρ=−0.41, p=0.041) and Symptoms scores (ρ=−0.40, p=0.038). TNF-α and FC were inversely associated with KOOS Symptoms scores (ρ=−0.38, p=0.027; ρ=−0.46, p=0.011, respectively), indicating greater symptom burden. Patients with IL-6 >5 pg/mL and TNF-α >10 pg/mL exhibited higher relative abundance of Romboutsia, Coprococcus and the Ruminococcus torques group. Lower IL-6 (<5 pg/mL) and TNF-α (<10 pg/mL) levels were associated with higher relative abundance of the short-chain fatty acid-producing genera Faecalibacterium, Roseburia and Subdoligranulum. Higher IL-10 concentrations (>10 pg/mL) were associated with higher abundance of Faecalibacterium, Roseburia, Blautia and Bifidobacterium. Stratification according to FC (<50, 50–250 and >250 μg/g) revealed progressive depletion of butyrate-producing bacteria, reduced microbial diversity and expansion of opportunistic taxa.

CONCLUSIONI

End-stage knee OA exhibits a distinct gut-derived inflammatory phenotype linking intestinal inflammation, microbial dysbiosis and increased inflammatory burden. These findings support the gut–joint axis as a biologically relevant mechanism underlying distinct microbiota-associated inflammatory profiles in OA.

B036

VALIDATION OF THE EPHA2- TARGETING A40S APTAMER IN PANCREATIC ADENOCARCINOMA CELLS AND EXTRACELLULAR VESICLES

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INTRODUZIONE

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, characterized by late diagnosis, rapid progression, and limited therapeutic options. The high mortality rate of PDAC is largely due to its silent progression, early metastatic dissemination, and intrinsic resistance to available therapeutic interventions. The identification of biomarkers associated with tumor aggressiveness and treatment resistance is therefore essential for the development of innovative diagnostic and therapeutic strategies. Among these, EphA2 receptor tyrosine kinase is frequently overexpressed in PDAC and has been associated with poor prognosis, metastatic dissemination, and drug resistance. A40s is a previously characterized RNA aptamer originally selected against glioblastoma stem cells and subsequently identified as a high-affinity ligand of EphA2.

METODI

Based on the overexpression of EphA2 in PDAC, we investigated the ability of A40s to recognize pancreatic cancer cells and their extracellular vesicles (EVs). Results obtained through RT-qPCR, FACS, ELONA, and immunofluorescence consistently demonstrated specific and reproducible binding to EphA2-expressing PDAC cells.

RISULTATI

Furthermore, the aptamer efficiently recognized EVs released by PDAC cells, confirming the presence and accessibility of its molecular target in both cellular and vesicular compartments. Overall, our findings extend the applicability of the A40s aptamer beyond glioblastoma and support its potential use as a molecular tool for the detection and targeting of EphA2-expressing pancreatic tumors and tumor-derived extracellular vesicles.

CONCLUSIONI

These results highlight A40s as a promising candidate for the development of novel diagnostic and therapeutic approaches in pancreatic cancer.

B037

INTEGRATIVE TRANSCRIPTOMIC AND MIRNA NETWORK ANALYSIS REVEALS MOLECULAR SIGNATURES OF CHEMORESISTANCE IN COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide. Although combined treatments based on 5-fluorouracil (5-FU), irinotecan (IRN), and oxaliplatin (OX) represent the standard of care for advanced CRC, therapeutic efficacy is frequently compromised by the emergence of drug resistance, ultimately leading to disease progression and poor outcomes. The regulatory landscape underlying chemoresistance in CRC remains incompletely characterized, limiting the development of predictive biomarkers and targeted therapeutic strategies. Accumulating evidence also suggests that aberrant microRNA (miRNA) expression contributes substantially to transcriptional perturbation in CRC, potentially responsible for the onset and maintenance of chemoresistant phenotypes.

METODI

Considering the role of transcriptional alterations and miRNAs as drivers of chemoresistance, we performed an integrative bioinformatic analysis of transcriptomic datasets derived from CRC cell lines sensitive or resistant to 5-FU, IRN and OX. Then, computational analyses were performed to identify dysregulated miRNAs associated with CRC progression and therapeutic failure and potentially involved in the transcriptional dysregulation associated with drug resistance.

RISULTATI

By combining differential gene expression profiles, pathway enrichment analysis and network-based approaches, we identified distinct drug-specific transcriptional meta-signatures associated with resistant phenotypes. Analysis of experimentally validated miRNA-target interactions revealed that hsa-miR-20a-5p, hsa-miR-183-5p, hsa-miR-182-5p, hsa-miR-503-5p, hsa-miR-145-5p, hsa-miR-143-3p, hsa-miR-375-3p and miR-195-5p are regulators of resistance-related gene expression signatures, as also confirmed by miRNA over-expression and silencing experiments on CRC cell lines using miRNA mimics and inhibitors, respectively.

CONCLUSIONI

Overall, these miRNAs may shape adaptive responses to chemotherapy by regulating key genes within the chemoresistance signatures. The convergence of their targets on pathways governing cell survival, apoptosis evasion, and therapy-induced cellular plasticity highlights a role for miRNA-mediated networks in driving drug resistance. Collectively, our findings provide a comprehensive epigenetic and transcriptomic background describing the molecular architecture of CRC chemoresistance and highlight candidate gene and miRNA signatures with prognostic and therapeutic relevance.

B038

IDENTIFICATION OF CIRCULATING AND TISSUE MIRNA SIGNATURES AS DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN UVEAL MELANOMA

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INTRODUZIONE

Uveal melanoma (UM) is the primary intraocular malignancy in adults and is characterized by high metastatic potential and poor prognosis. Therefore, the identification of reliable diagnostic and prognostic biomarkers is crucial for improving early detection and patient management. In this context, circulating and tissue microRNAs (miRNAs) represent promising epigenetic regulators involved in tumor development and progression. This study aimed to evaluate the diagnostic and prognostic potential of hsa-miR-199a-5p, hsa-miR-508-3p, and hsa-miR-514a-3p in UM through liquid biopsy, FFPE tissue analyses, and integrative bioinformatic approaches.

METODI

Serum samples from 47 UM patients and 10 healthy donors, together with 11 UM FFPE tissue samples and 3 normal choroidal FFPE tissues, were analyzed through droplet digital PCR (ddPCR) to evaluate miRNA expression levels. In parallel, bioinformatic analyses were performed using miRWalk for target gene prediction, STRING for protein–protein interaction networks, GO Panther for functional annotation, miRCancerdb for correlation analysis, and TCGA-UVM data for clinical and prognostic associations.

RISULTATI

ddPCR analysis revealed a significant overexpression of hsa-miR-199a-5p expression levels in UM patients compared to healthy donors with moderate diagnostic accuracy as indicated by ROC analysis. In FFPE tissues, hsa-miR-199a-5p was confirmed to be significantly up-regulated in tumor samples, whereas hsa-miR-508-3p and hsa-miR-514a-3p were significantly down-regulated compared to normal choroidal tissues according to the same data observed in the TCGA-UVM cohort .

Bioinformatic analyses identified 30 genes commonly targeted by all three miRNAs and 87 genes shared by the two down-regulated miRNAs. Functional enrichment analyses indicated involvement in key pathways related to cell signaling, proliferation, and tumor progression, including EGFR, Wnt, and cadherin signaling pathways.

CONCLUSIONI

This study highlights the potential role of hsa-miR-199a-5p as a non-invasive diagnostic biomarker for uveal melanoma and suggests that hsa-miR-508-3p and hsa-miR-514a-3p may have prognostic relevance in tumor progression. Bioinformatics analyses confirm the involvement of these miRNAs in regulatory networks associated with UM pathogenesis, supporting further studies on larger cohorts to validate their diagnostic and prognostic utility.

B039

SELECTIVE TARGETING OF FOLATE RECEPTOR-POSITIVE CANCER CELLS WITH MEBENDAZOLE-LOADED FOLATE-FUNCTIONALIZED MESOPOROUS SILICA NANOPARTICLES

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INTRODUZIONE

Breast cancer remains the most diagnosed malignancy among women worldwide. Therapeutic resistance in estrogen receptor-positive (ER^+) tumors and the aggressive nature of triple-negative breast cancer (TNBC) highlight the need for novel therapeutic strategies. Mebendazole (MBZ), an FDA-approved anthelmintic drug, has emerged as a promising candidate for drug repurposing in oncology due to its antitumor activity; however, its clinical use is limited by poor oral bioavailability. To overcome this limitation, we developed a folate-functionalized mesoporous silica nanosystem (FOL-MSN-MBZ) for selective delivery of MBZ to folate receptor-positive (FR^+) breast cancer cells.

METODI

FOL-MSN-MBZ was synthesized by covalently grafting folic acid onto MSN surfaces, followed by MBZ loading into the nanopores via a two-step impregnation process (drug loading: 8.80%). Its anticancer activity was evaluated in FR^+ breast cancer cell lines, including MCF-7, tamoxifen-resistant MCF-7 (TamR), TamR T47D, MDA-MB-231, and MDA-MB-468, as well as in FR^- non-tumoral cells, using proliferation and clonogenic assays. Pro-apoptotic effects of free MBZ and FOL-MSN-MBZ were analyzed by TUNEL assay, and the efficacy of the nanosystem was further assessed in 3D spheroid models.

RISULTATI

FOL-MSN-MBZ induced selective cytotoxicity in FR^+ breast cancer cells while sparing FR^- non-tumoral cells. In contrast to free MBZ, which showed non-selective toxicity, the nanosystem preserved antitumor efficacy while improving tumor selectivity. Moreover, FOL-MSN-MBZ reduced clonogenic potential and impaired spheroid growth and viability in 3D models. TUNEL analysis confirmed that encapsulated MBZ retains the pro-apoptotic activity of the free drug in FR^+ breast cancer models.

CONCLUSIONI

Our results indicate that FOL-MSN-MBZ enables targeted delivery of MBZ, inducing cytotoxicity in FR^+ tumor cells while sparing normal FR^- cells, thereby reproducing the antitumoral effects of free MBZ with enhanced tumor specificity. These findings support FOL-MSN-MBZ as a promising nanomedicine strategy to improve MBZ therapeutic index and to address aggressive and resistant breast cancer subtypes. Supported by NextGenerationEU - PNRR e PRIN - Project Title "Drug repositioning as a safer and sustainable way to fight hard-to-treat cancer" -P2022T7FXB.

B040

SPATIAL TRANSCRIPTOME ANALYSIS OF SCAFFOLD FREE PATIENT DERIVED ORGANOIDS FROM GLIOMAS AND MENINGIOMAS

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INTRODUZIONE

Gliomas and meningiomas are primary central nervous system tumors with distinct biology and prognosis. Despite therapeutic advances, high-grade gliomas and atypical/anaplastic meningiomas remain largely incurable, highlighting the need for predictive patient-derived ex vivo models that faithfully recapitulate tumor heterogeneity and support precision oncology. In this study we aimed to generate and characterize scaffold-free patient-derived organoids (PDOs) from both gliomas and meningiomas as a versatile preclinical platform.

METODI

We established scaffold-free organoid cultures from glioma and meningioma samples and performed a comprehensive characterization of the resulting models. Culture efficiency was assessed across tumor types, and the organoids were analyzed for preservation of molecular features, cellular composition, vascular structures, and immune components. In addition, we applied high-resolution spatial transcriptomics to paired PDOs and matched parental glioblastoma tissue to evaluate the preservation of cellular state and tissue architecture.

RISULTATI

Using this approach, we achieved a culture generation success rate of more than 60–70%, including in slow-growing tumors. Our analyses showed that the PDOs retained key molecular features of the parental tissue and preserved the major cellular populations present in the original tumors. Notably, vascular structures and immune components were maintained for up to one month after culture generation. Spatial transcriptomics further confirmed that the scaffold-free organoid system preserved the structural organization of the parental glioblastoma tissue and recapitulated its cellular architecture.

CONCLUSIONI

Collectively, these findings show that scaffold-free organoid culture can be successfully applied across different brain malignancies while preserving tumor complexity and key microenvironmental components. This system therefore represents a robust preclinical model for drug screening and precision medicine, and for studying interactions between tumor cells and their niche.

B041

TARGETING ZMAT3 TO COUNTERACT SENESCENCE-DRIVEN ADIPOSE TISSUE DYSFUNCTION: A TRANSLATIONAL APPROACH

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INTRODUZIONE

Senescent cell accumulation contributes to adipose tissue (AT) dysfunction in aging, obesity and type 2 diabetes (T2D), promoting inflammatory and metabolic dysfunction through the senescence-associated secretory phenotype (SASP). We previously identified ZMAT3 as a mediator of senescence in adipose precursor cells from first-degree relatives (FDRs) of T2D patients and found it upregulated in adipocytes from obese and T2D subjects. Here, we tested whether siRNA-mediated Zmat3 silencing could attenuate senescence-associated alterations in metabolic disorders, supporting RNA-based targeting strategies.

METODI

Doxorubicin-induced adipocyte senescence was analyzed by qRT-PCR and Western blot for molecular markers, multiplex immunoassay for SASP, extracellular flux analysis for mitochondrial function, and RNA-seq for mechanistic profiling. FDR-derived human adipocytes were used to assess lipid nanoparticle-mediated siRNA delivery.

RISULTATI

Doxorubicin promoted a senescence-associated response in adipocytes, characterized by increased ZMAT3 and senescence markers at mRNA/protein levels. This response was accompanied by SASP-like inflammatory alterations and impaired adipocyte function, reflected by reduced lipid accumulation, decreased adipogenic/metabolic marker expression and defective insulin signaling, consistent with AT dysfunction. Bioenergetic profiling revealed impaired energy homeostasis, with reduced basal respiration and mitochondrial ATP production, increased glycolytic ATP production and reduced total ATP levels.
Zmat3 silencing attenuated the doxorubicin-induced senescence phenotype, reduced senescence markers and improved mitochondrial function, increasing basal respiration, mitochondrial ATP production and total ATP levels. Cytokine profiling supported partial mitigation of the SASP-like inflammatory response, mainly driven by reduced IL-6 secretion. In FDR-derived human adipocytes, lipid nanoparticle-mediated siRNA delivery was well tolerated and effectively silenced ZMAT3, supporting in vivo studies.

CONCLUSIONI

Our data identify ZMAT3 as a translational target in senescence-driven AT dysfunction and support RNA-based ZMAT3 targeting to mitigate inflammatory and bioenergetic alterations underlying AT remodeling in obesity and T2D.

B042

TARGETING NADH HOMEOSTASIS THROUGH GPD2 INHIBITION AND METABOLIC STRESS INDUCES TUMOR REGRESSION IN COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) is characterized by metabolic reprogramming to sustain tumor growth. Mitochondrial glycerol-3-phosphate dehydrogenase (GPD2), a key enzyme in the glycerophosphate shuttle, emerges as a pivotal regulator of redox balance in CRC.

METODI

Using virtual screening, we identified a novel GPD2 inhibitor, termed G2i. We used CRC cells lacking GPD2 and/or treated with G2i to perform proliferation, metabolic and bioenergetic assays. APC deleted mice were treated with G2i and after one month, mice were sacrificed, intestines explanted and processed for histological and molecular analyses. MC38 allografted mice were treated with G2i alone and combined with LDH inhibitor or intermittent fasting (IF) protocols. After two weeks tumor masses were processed as above.

RISULTATI

Using publicly available datasets and tumor samples, we found that GPD2 mRNA and protein levels are significantly upregulated in colorectal cancer (CRC) compared to normal mucosa. To investigate the functional consequences of this overexpression, we genetically or pharmacologically inhibited GPD2, using shRNAs or our newly identified inhibitor G2i. GPD2 inhibition impaired tumor growth both in vitro and in CRC mouse models by disrupting mitochondrial NADH oxidation, leading to reductive stress, glycerol-3-phosphate accumulation, decreased ATP levels, and impaired cellular respiration. These effects were rescued by restoring NADH oxidation, confirming that redox imbalance is the key vulnerability induced by GPD2 blockade.
Given that lactate dehydrogenase (LDH) serves as a compensatory cytosolic NADH sink, we next evaluated the effect of dual inhibition. Co-targeting GPD2 and LDH triggered redox collapse and halted CRC growth both in vitro and in vivo. To further exacerbate metabolic stress, we implemented intermittent fasting (IF) protocols, which limit nutrient availability. IF synergized with G2i, amplifying NADH accumulation and energetic failure, thereby enhancing tumor suppression.

CONCLUSIONI

Together, our findings position GPD2 as a druggable node in CRC redox metabolism. GPD2 inhibition induces synthetic redox stress limiting mitochondrial function and tumor viability. This effect can be enhanced by combining targets of compensatory NADH sinks or nutrient restriction strategies.

B043

COULD PLATELET-DERIVED LONGEVITY SIGNATURES UNDERLIE ANTAGONISTIC PATHOGENIC PLEIOTROPY IN ESSENTIAL THROMBOCYTHEMIA? EVIDENCE FROM PLATELET FACTOR 4 AND BPIFB4

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INTRODUZIONE

Long-lived individuals (LLIs) defy the conventional trajectory of aging characterized by attenuated inflammaging and preserved immune balance, achieved by a favorable platelet secretome, including BPIFB4, a well stated biomarker of healthy aging. Essential thrombocythemia (ET) is a disease characterized by elevated platelet count and expected thrombotic risk. Nevertheless, patients maintain near-normal life expectancy. Thus, we investigated whether platelet-derived health span-associated factors (platelet-factor 4 and BPIFB4) are responsible for health span in ET patients recapitulating successful aging of LLIs, explaining the phenomenon of Antagonistic Pathogenic Pleiotropy (APaP).

METODI

We assessed clinical data from ET patients (n=21; mean age 50) compared to age-matched healthy controls (HC, n=30: mean age 50) and LLIs (n=18, age >95). PF4 and BPIFB4 plasma levels were evaluated by ELISA and platelets function by aggregometry analysis. CD47, a marker of platelet turnover, was also analyzed by flow cytometry.

RISULTATI

LLIs showed higher levels of PF4 compared to aged cohort (1406,3±202,1 vs 330,81±100,1ng/ml; p<0,0001). Similarly, ET patients showed elevated PF4 and BPIFB4 levels compared to HC (respectively 1688,79±371,2 vs 291,5±212,3 ng/ml, p<0,0001; 348,1±177,7 vs 112,3±78,9 pg/ml, p<0,0001) consistent with the increased platelet count. The platelet surface expression of CD47 - a don't eat me signal - is lower in ET compared to HC (1611,9±480 vs 2118,25±149,9 expressed as GeoMean; p<0,001). Functionally, ADP response in aggregometry studies is impaired in ET patients (27,8±7,8% vs 68±3,6%, p<0,0001).

CONCLUSIONI

Our preliminary data support the APaP framework in ET: the pathological burden of ET patients appears counterbalanced by sustained overproduction of pro-longevity factors PF4 and BPIFB4, mirroring key molecular traits that distinguish LLIs from general population. CD47 downregulation on ET platelets is proposed as a first mechanistic hypothesis, linking enhanced platelet turnover to amplified release of anti-aging mediators. While exploratory, these findings lay the ground for future studies aimed at explaining whether a pathological process may have favorable compensative effects although not strictly correlated to physiopathological conditions.

B044

ADVANCED BIOLUMINESCENCE MOLECULAR IMAGING REVEALS HIDDEN CENTRAL NERVOUS SYSTEM TROPISM IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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INTRODUZIONE

Visceral leishmaniasis (VL) is a systemic parasitic disease primarily affecting the spleen, liver and bone marrow. Increasing evidence suggests that Leishmania parasites may also reach the central nervous system (CNS), a compartment whose pathological significance remains poorly understood. Detecting low parasite burdens within deep tissues is challenging using conventional approaches, limiting the identification of hidden tissue reservoirs and parasite dissemination pathways. Here, we evaluated next-generation bioluminescence imaging (BLI) to investigate CNS involvement during experimental VL.

METODI

A Leishmania donovani line expressing the highly-sensitive reporter Akaluc was generated and validated through molecular characterization, growth and infectivity analyses. Its performance was compared with click beetle and conventional firefly luciferases. BALB/c and C57BL/6 mice were monitored longitudinally by whole-body and cranial-region BLI. Ex vivo imaging was performed on major organs and CNS-associated tissues. Viable parasites were assessed by limiting dilution and molecular analyses. The feasibility of a reporter-based immunodetection strategy for histopathological parasite identification was also investigated.

RISULTATI

The Akaluc-expressing parasite line displayed stable reporter expression without affecting parasite growth or infectivity. In vivo imaging enabled sensitive monitoring of infection and consistently detected cranial-associated bioluminescent signals. Compared with other luciferase systems, Akaluc showed superior sensitivity for detecting infection in anatomically challenging regions. Ex vivo analyses confirmed bioluminescent signals in CNS-associated tissues, while viable parasites were recovered from brain samples, supporting CNS involvement during experimental infection. Reporter-based immunodetection was validated in cultured parasites and infected cells and further explored for histopathological parasite identification in FFPE tissues.

CONCLUSIONI

Collectively, these findings highlight advanced bioluminescence imaging as a powerful translational tool for investigating overlooked tissue reservoirs and tissue tropism in VL. Improved detection of parasites within non-classical anatomical sites may facilitate future studies of disease pathogenesis, dissemination dynamics and the role of hidden tissue compartments in infection.

B045

FROM METABOLIC DYSFUNCTION TO MORTALITY: CLINICAL INSIGHTS FROM THE TRIGLYCERIDE-GLUCOSE INDEX IN HOSPITALIZED OLDER ADULTS

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INTRODUZIONE

Insulin resistance is a major driver of metabolic dysfunction and a key contributor to the development of age-related diseases. The triglyceride-glucose (TyG) index, calculated from routine fasting glucose and triglyceride measurements, has emerged as a simple and cost-effective surrogate marker of insulin resistance. Its clinical and prognostic relevance has been increasingly explored in metabolic disorders, particularly type 2 diabetes, but evidence in hospitalized older adults remains limited. This study aimed to investigate the association between TyG index, metabolic comorbidities, and survival in a large cohort of geriatric inpatients.

METODI

A retrospective analysis was conducted on 5,954 hospital admissions corresponding to 4,852 unique patients aged ≥65 years admitted to IRCCS INRCA hospitals. The TyG index was calculated as Ln [fasting triglycerides (mg/dL) × fasting plasma glucose (mg/dL)/2]. Clinical, demographic, and laboratory data were collected from electronic health records. The relationship between TyG index, metabolic comorbidities, and survival was investigated through descriptive and inferential statistical analyses.

RISULTATI

TyG index showed significant associations with demographic and clinical characteristics of the study population. Patients with diabetes exhibited significantly higher TyG values than non-diabetic individuals (8.82 vs 8.53, $p<0.001$), with the highest values observed in patients with diabetes-related complications (8.96 vs 8.57, $p<0.001$). Increased TyG values were also associated with peripheral vascular disease (8.74 vs 8.58, $p<0.001$), renal disease (8.62 vs 8.57, $p=0.009$), dementia ($p=0.048$), and metastatic cancer ($p=0.014$). Moreover, survival analyses showed progressively reduced survival across increasing TyG tertiles.

CONCLUSIONI

TyG index is associated with metabolic comorbidity burden and poorer survival in hospitalized older adults. As an inexpensive and readily available surrogate marker of insulin resistance, it may represent a valuable tool for metabolic risk stratification in geriatric clinical practice.

B046

GENETIC DETERMINANTS OF MCI-TO-ALZHEIMER'S DISEASE CONVERSION IN TYPE 2 DIABETIC PATIENTS

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INTRODUZIONE

Mild cognitive impairment (MCI) represents a transitional stage between normal aging and dementia and is associated with an increased risk of progression to Alzheimer's disease (AD). Although the molecular mechanisms underlying this transition remain incompletely understood, inflammation and metabolic dysfunction are increasingly recognized as key contributors. In particular, Type 2 Diabetes (T2D), a chronic inflammatory and metabolic disorder, has emerged as a significant risk factor for cognitive decline and dementia. Furthermore, genetic variation in inflammation- and metabolism-related pathways may contribute to individual susceptibility to disease progression. This study investigated the association between specific genetic variants and MCI-to-AD conversion in individuals with T2D, aiming to identify potential genomic predictors of AD onset.

METODI

Whole-exome sequencing (WES) was performed on peripheral blood gDNA from 200 MCI patients stratified to T2D status and conversion to AD: T2D−/C− (n = 62), T2D−/C+ (n = 82), T2D+/C− (n = 27), and T2D+/C+ (n = 29). Sequencing data were processed using the GATK Best Practices workflow, including variant calling, joint genotyping, quality recalibration, and filtering of low-quality genotypes prior to comparative analyses.

RISULTATI

In agreement with the established association between APOE-ε4 and AD, APOE-ε4 carrier status was significantly linked to MCI-to-AD conversion in T2D− patients and, to a lesser extent, in the overall cohort. Notably, this association was not observed in T2D+ patients, suggesting that additional factors may drive disease progression in this subgroup. Exploratory analyses of variants in genes related to clonal hematopoiesis, inflammasome signaling, AD susceptibility, and metabolic-neurodegenerative pathways identified enrichment patterns associated with MCI-to-AD conversion in T2D+ patients, including alterations in inflammation- and metabolism-related genes potentially involved in diabetes-dependent neurodegeneration.

CONCLUSIONI

Our findings support the contribution of distinct inflammation- and metabolism-related genetic factors to AD progression in T2D patients, suggesting diabetes-specific mechanisms of neurodegeneration.

This work was supported by the Italian Ministry of Health under the PNRR Mission 6, Project PNRR-MCNT2-2023-12377692, CUP I53C23002660005

B047

THE CONSEQUENCES OF CNBP REDUCED EXPRESSION IN DM2 PATHOGENESIS

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INTRODUZIONE

Myotonic dystrophy type 2 (DM2) is an autosomal dominant disorder that primarily affects skeletal muscle, leading to muscle fiber degeneration and dysfunction. DM2 is caused by a CCTG repeat expansion within intron 1 of the CNBP (Cellular Nucleic acid-Binding Protein) gene, although the precise pathogenic mechanisms remain poorly understood. This study aims to determine whether CNBP expression is reduced in the context of DM2 and to explore the functional consequences of CNBP depletion on muscle physiology and performance.

METODI

We analyzed samples derived from DM2 patients and performed in vivo studies using CNBP transgenic mice and Drosophila models to evaluate the effects of CNBP depletion on muscle structure and function.
Additional experiments in murine and human myoblasts, as well as in transgenic models, were carried out to investigate the molecular consequences of CNBP loss.

RISULTATI

We found that CNBP expression is reduced in DM2 patient-derived myoblasts carrying CCTG repeat expansions. Further studies in myoblast cell lines, transgenic mice, and Drosophila models confirmed that CNBP depletion significantly impairs muscle function. Specifically, CNBP loss led to severe locomotor defects in Drosophila, while heterozygous CNBP knockout mice developed late-onset motor deficits and muscle atrophy.
Transcriptomic analyses of CNBP-depleted myoblasts revealed a marked downregulation of genes involved in muscle cell differentiation, accompanied by upregulation of autophagy-related genes. Autophagy activation was observed across both in vitro and in vivo models. Notably, in Drosophila, RNAi-mediated silencing of ATG7, a key autophagy regulator, rescued the locomotor defects induced by CNBP loss.

CONCLUSIONI

In summary, our findings suggest that CNBP haploinsufficiency leads to aberrant activation of autophagy via TFEB, contributing to muscle degeneration and functional impairment in DM2. This study provides novel insights into disease pathogenesis and identifies autophagy modulation as a potential therapeutic target in DM2.

B048

RNA-LOADED NANOMEDICINE TO MODULATE LOCAL INTESTINAL INFLAMMATION IN INFLAMMATORY BOWEL DISEASE

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INTRODUZIONE

Inflammatory Bowel Diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract, with Crohn's disease and ulcerative colitis representing two major clinical phenotypes. Current therapeutic strategies show only moderate efficacy and are often associated with significant systemic adverse effects, compromising patients' quality of life. Thus, IBD treatment remains a major unmet clinical need. We aim to restore intestinal homeostasis by using RNA-loaded lipid nanoparticles (LNPs, under patent consideration) to deliver therapeutic nucleic acids designed to silence selectively the intestinal production of pro inflammatory cytokines.

METODI

As a preliminary evaluation, Cy5-labelled LNPs containing siRNAs targeting a non coding transcript or the selected pro inflammatory cytokine were tested on mucus-secreting human intestinal monolayers based on a mixed population of Caco-2/HT29-MTX cells, on macrophage-like THP-1 cells, and in a triple co-culture model in which epithelial cells and THP-1 cells were seeded on the opposite sides of the same insert. Cell viability, transepithelial electrical resistance (TEER), nanoparticle internalization (through confocal microscopy), and gene expression were assessed under control or pro-inflammatory conditions (mimicked by the exposure to LPS + IFNγ + TNFα). In parallel, the same model was treated with LNPs containing GFP mRNA.

RISULTATI

After 24h, RNA-LNPs were efficiently internalized by the intestinal monolayer in both co-culture types, as indicated by high Cy5 fluorescence and GFP expression. The nano-system did not affect epithelial viability or TEER. THP-1 cells also internalized RNA-LNPs and showed reduced expression of the target pro-inflammatory cytokine, although with a decrease in viability in monoculture that was not evident in co-culture. Importantly, in the co-culture model, RNA-LNPs still modulated the inflammatory target.

CONCLUSIONI

These preliminary findings indicate that RNA-LNPs are biocompatible with in vitro intestinal models and effectively modulate inflammatory pathways. If confirmed and validated in more complex in vitro and in pre-clinical models, this approach may pave the way for the oral delivery of RNA-based nanotherapeutics in IBD.

Acknowledgment: GT is supported by AIRC, Italy.

B049

IDENTIFICATION OF FITNESS-ASSOCIATED LNCRNAS INTERACTING WITH ESTROGEN RECEPTOR α IN OVARIAN CANCER CELL MODELS.

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INTRODUZIONE

Ovarian cancer(OC) represents a significant global health burden, with a high incidence and still a leading cause of cancer-related death in women worldwide. Standard treatment includes cytoreductive surgery followed by platinum-based chemotherapy; however, recurrence rates remain high and overall survival poor. In this context the estrogen receptor alpha (ERα) received a lot of interest for its connection to female malignancies with breast tumor. ERα is now recognized as a non-canonical RNA-binding protein capable of directly binding RNAs, influencing chromatin organization, and regulating transcriptional programs beyond classical estrogen response elements. Emerging evidence suggests that non-coding RNAs, particularly long non-coding RNAs (lncRNAs)play pivotal roles in OC tumor proliferation, chemoresistance, DNA repair regulation, and metastasis. For this reason, we investigated the lncRNAs that interact with ERα, regulating its transcriptional program.

METODI

PEO1, PEO4, PEO14, and OVCAR3 OC cell lines were characterized in this study. Particularly, first we investigated the transcriptome profiles (RNA-seq) to define the lncRNAs mainly expressed in these models. Then, a native nuclear Immunoprecipitation of ERα coupled to RNA sequencing (RIP-Seq) was performed in PEO1 and PEO4 OC cell lines.

RISULTATI

RNA-seq data of PEO1, PEO4, PEO14 and OVCAR3 OC cell lines were analyzed to identify expressed lncRNAs In these models. On the other hand, to obtain ERα interacting lncRNAs, RIP-seq experiments were done in PEO1and PEO4, representing sensitive and resistant cell lines to platinum-based therapy. To focus on fitness-related lncRNAs we analyzed open access datasets. Besides, literature was skimmed for all available data on proved essential lncRNAs and their functional studies in ovarian cancer.

CONCLUSIONI

Overall, our results defined actionable lncRNAs interacting with ERα and, some of them, have defined roles in tumor progression representing putative new therapeutic targets to overcome resistance mechanisms.

B050

CYTOKINE-ASSOCIATED IMMUNE REGULATION, CXCR4 EXPRESSION, AND MACROPHAGE POLARIZATION IN ALTEPLASE-ASSOCIATED ANGIOEDEMA

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INTRODUZIONE

Alteplase-associated angioedema (ISAE) is a rare but serious complication of thrombolysis for acute ischemic stroke, whose underlying immunological mechanisms remain unknown. This study aimed to examine whether acute ISAE is accompanied by specific alterations in cytokines and plasma-induced modulation of macrophage polarization and CXCR4 expression.

METODI

Eight alteplase-treated ischemic stroke patients were analyzed: ischemic stroke controls without angioedema (ISC, n=4) and patients with ISAE (n=4) evaluated at the acute episode and following remission. A bead-based multiplex immunoassay was used to quantify plasma MCP-1, IL-6, IL-1β, IL-10, IL-23, and IL-33. Macrophages were differentiated from healthy-donor PBMC for 7 days in culture medium supplemented with plasma from ISC or acute ISAE or remission ISAE samples. Flow cytometry was used to assess macrophage subset expression and CXCR4 levels.

RISULTATI

Cytokine profiles were not significantly different between ISC and ISAE remission samples. In longitudinal ISAE analysis, the level of IL-10 was significantly higher in acute angioedema than in remission ($p<0.01$), while levels of IL-1β, IL-6, IL-23, IL-33, and MCP-1 had modest or variable changes that were not statistically significant. The conditioned macrophages with remission plasma from ISC and ISAE showed comparable M2/M1 ratios, and plasma from acute ISAE had a significantly higher M2/M1 ratio compared to remission plasma ($p<0.01$), demonstrating that the plasma skewed the macrophages towards an M2-like phenotype. Total macrophage proportions and the distribution of M1-like (CD14+CD86+) and M2-like (CD206+CD163+) subsets were broadly comparable between ISC and remission groups. The acute ISAE plasma enhanced CXCR4 expression in M1-like macrophages compared to remission and control plasma, although M2-like macrophages remained stable.

CONCLUSIONI

Acute ISAE is associated with a transient IL-10-dominant cytokine profile, plasma-driven skewing towards M2-like macrophage phenotype, and selective upregulation of CXCR4 on M1-like macrophages. These preliminary findings suggest a dynamic immune-regulatory response in acute angioedema and provide a foundation for future research into immune-cell mechanisms that may underlie the development and resolution of ISAE.

B051

TARGETING AMINO ACID TRANSPORTOME TO OVERCOME CASTRATION RESISTANT PROSTATE CANCER

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INTRODUZIONE

Progression to castration-resistance remains a major clinical challenge in prostate cancer (PCa) and is largely associated with metabolism rewiring and microenvironment support. Castration resistant cells exhibit increased dependency on glutamine and asparagine, however this vulnerability is mitigated by co-culture with stromal cells, pointing to a role for membrane transport in the crosstalk (manuscript under review) In this context, the contribution of membrane transporters (Solute Carriers, SLCs) in PCa progression is poorly defined. We hypothesize that specific SLCs are required for PCa survival during AR blockade.

METODI

To approach in vivo conditions, androgen independent (PC-3, DU145) and androgen dependent (LNCaP, VCaP) PCa cell lines were cultured in Plasmax™, an advanced medium containing physiological metabolite levels, and treated with the AR antagonist enzalutamide (Enz) with or without amino acid transport inhibitors. LNCaP cells were also co-cultured with primary prostate-derived normal fibroblasts (pNF) or prostate cancer-associated fibroblasts (pCAF).

RISULTATI

Androgen-independent cells exhibited higher expression of several amino acid transporters, such as SLC1A5, SLC7A5, SLC7A11, SLC38A1, SLC38A2, SLC38A5 and SLC38A7 than androgen-dependent cells. Inhibition of the transporter SLC7A11 with sulfasalazine markedly inhibited PC-3 and DU145 cell proliferation, whereas the inhibition of the glutamine transporter SLC1A5 by V-9302 reduced the growth of all the PCa lines. Notably, V-9302 increased Enz sensitivity of PC-3 cells, while the inhibitor, along with the SLC38A5 inhibitor His and the SLC38A2 inhibitor MeAIB, had the same effect in DU145 cells. LNCaP cells maintained growth upon Enz treatment when co-cultured with pNF or pCAF.

CONCLUSIONI

Overall, these preliminary findings indicate that androgen insensitive phenotype is associated with an extensively rewired transportome, prompting a genome-wide analysis of SLC role in castration-resistant PCa. A CRISPR Cas9 SLC knockout screening is, therefore, underway to prioritize candidate transporters associated with the castration-resistant phenotype. The identified targets will be functionally validated in advanced co-culture models of PCa and stromal cells.
Acknowledgment: GT is supported by AIRC, Italy.

B052

MULTIPLE MYELOMA GLUTAMINE ADDICTION SHAPES A PRO-TUMOR BONE MARROW NICHE

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INTRODUZIONE

Multiple myeloma (MM) is a glutamine (Gln)-addicted cancer characterized by malignant plasma cell growth in the bone marrow (BM) and severe osteolytic bone disease. MM cells alter BM plasma amino acid composition, reducing Gln and increasing glutamate (Glu), thus generating a metabolic environment that impairs osteogenic differentiation of mesenchymal stromal cells (MSCs) and promotes osteoclastogenesis. Since MM BM is also enriched in adipocytes and tumor-associated macrophages, we investigated how MM-driven metabolic alterations affect adipogenesis and macrophage polarization.

METODI

The cell models exploited were primary human BM MSC from either healthy donors (HD) or MM patients, monocyte-derived macrophages (MDM), from monocytes of HD peripheral blood, and human MM cell lines (HMCL).

RISULTATI

HMCL-conditioned media ([Gln]=0.3 mM) increased adipogenic markers in both HD- and MM-MSCs; this effect was blunted by Gln supplementation. Consistently, Gln deprivation enhanced lipogenesis and adipogenic markers in MSC. Early adipogenesis was associated with nutritional stress and reduced intracellular Gln, Glu and asparagine (Asn), whereas leucine and isoleucine were unchanged. Glutamine synthetase (GS) expression increased, while glutaminase and the Gln transporters SNAT2 and SNAT5 decreased, suggesting low dependence on extracellular Gln of adipocytes. GS silencing impaired adipogenesis in Gln-fed MSCs, indicating a functional role for GS induction. Notably, stromal cells, but not malignant plasma cells, were strongly GS-positive in MM bone biopsies. Asn supplementation inhibited adipogenic differentiation under Gln deprivation, counteracting ROS accumulation and the SOD1/CAT upregulation induced by Gln shortage, consistent with an antioxidant-mediated effect. MDM co-cultured with HMCL upregulated GS, the Glu transporter EAAT1, and M2 markers. Also, MDM supported MM cell growth under Gln shortage, an effect hindered by GS inhibition.

CONCLUSIONI

Our results uncover a thus far uncharacterized Gln/Glu driven metabolic crosstalk in MM BM, that skews MSC differentiation from osteogenesis to adipogenesis and contribute to macrophage M2 polarization. Asn supplementation and GS-inhibition counteract these effects, pointing to potential therapeutic strategies to limit MM progression and pathological BM remodeling.

B053

A RAS FAMILY MEMBER MEDIATES ERK5 ACTIVATION IN RESPONSE TO THE INHIBITION OF ERK1/2 SIGNALING PATHWAY

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INTRODUZIONE

Malignant melanoma is one of the most aggressive cancers, with a rising incidence in the last decades. The Mitogen Activated Protein Kinase (MAPK) family, in particular ERK1/2 and ERK5 pathways, plays a key role in tumor growth and progression, including in melanoma. In BRAFV600E-mutant patients, targeted therapies against BRAF or MEK1/2 have significantly improved survival, especially when combined with immunotherapy. However, resistance mechanisms frequently limit their efficacy. Preclinical evidence suggests that RAS-RAF-MEK1/2-ERK1/2 inhibition may result in ERK5 activation, likely as a compensatory mechanism to support tumor growth. In this work, we identified a small Ras-family GTPase (here referred to as X-RAS) as a key mediator of this adaptive response in BRAFV600E-mutant melanoma.

METODI

BRAFV600E-mutated A375 and Sk-Mel-5 melanoma cells were silenced for X-RAS using a specific siRNA or treated with non-targeting siRNA (siNT) as control. HEK-293T cells were transfected with plasmids containing ERK5, a constitutively active form of X-RAS (X-RAS QL) and pCDNA3 as control. Small-molecule inhibitors used included BRAF inhibitor (vemurafenib), MEK1/2 inhibitor (trametinib), ERK1/2 inhibitor (SCH-772984) and pan-RAS inhibitor (RMC-6236).

RISULTATI

Using A375 and Sk-Mel-5 cells, we found that targeting BRAF–MEK1/2 and ERK1/2, respectively with vemurafenib plus trametinib or SCH-772984, enhanced ERK5 phosphorylation and upregulated X-RAS expression. Interestingly, X-RAS silencing prevented ERK5 activation and potentiated the pro-apoptotic effects of ERK1/2 inhibition. Moreover, the overexpression of the constitutively active X-RAS mutant increased ERK5 phosphorylation, supporting a role of X-RAS in regulating ERK5 activation following ERK1/2 pathway inhibition. Treatment with the pan-RAS inhibitor RMC-6236 dose-dependently reduced melanoma cell viability, and its combination with BRAF–MEK1/2–ERK1/2 inhibitors more effectively impaired both cell viability and 3D tumor growth compared with single agents.

CONCLUSIONI

Collectively, our findings established X-RAS as a critical regulator of ERK5 activation upon ERK1/2 pathway inhibition and a potential therapeutic target to overcome resistance in BRAFV600E-mutant melanoma.

B054

THE PIN1-WWP2 ANTAGONISM REGULATES NOTCH3 STABILITY AND PLATINUM RESISTANCE IN HIGH-GRADE SEROUS OVARIAN CANCER

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INTRODUZIONE

High-grade serous ovarian cancer (HGSOC) is the deadliest gynecologic malignancy worldwide. Despite maximal cytoreductive surgery and platinum-based chemotherapy, most patients relapse within two years with platinum-resistant disease. Among the pathways implicated in chemoresistance, Notch3 (N3) is frequently overexpressed in ovarian cancer, and it is associated with poor outcomes. Two regulators of N3 signaling have emerged as promising therapeutic targets in HGSOC: Pin1, a peptidyl-prolyl isomerase that enhances N3 oncogenic activity, and WWP2, an E3 ubiquitin ligase that selectively restrains N3 signaling. Overall, this study aims at investigating the unrecognized N3-Pin1-WWP2 axis to identify mechanisms controlling N3 stability.

METODI

1. lentiviral transductions to stably over-express doxycycline-inducible shRNA-Pin1; 2. mechanistic studies such as transient transfections, ubiquitination and protein stability assays, co-immunoprecipitation; 3. mass-spectrometry-based proteomics to unravel WWP2 phosphosites; and 4. molecular docking and dynamics simulations.

RISULTATI

Pin1 promoted N3 intracellular domain (N3ICD) stability through a dual effect on WWP2. In Pin1-deficient HEK293T cells, restoration of Pin1 disrupted the N3ICD-WWP2 interaction while favoring Pin1 binding to N3ICD, whereas mutation of four Pin1 consensus motifs within N3ICD restored WWP2 association, indicating that Pin1 limits WWP2 access to N3. Consistently, WWP2 overexpression reduced N3ICD stability and increased both mono- and polyubiquitination, while Pin1 re-expression abolished these effects, showing that Pin1 counteracts WWP2-dependent N3 degradation. We also identified six putative Pin1-binding motifs in WWP2 and confirmed Pin1-WWP2 interaction, suggesting that Pin1 may maintain WWP2 in an autoinhibited state. Together, these findings support a model in which Pin1 stabilizes N3ICD by antagonizing WWP2, thereby sustaining oncogenic signaling and promoting platinum resistance.

CONCLUSIONI

These findings identify the N3-Pin1-WWP2 axis as a post-translational mechanism underlying N3-driven chemoresistance in HGSOC and support Pin1 inhibition as a potential therapeutic strategy for platinum-resistant disease.

B055

TARGETING C1858T VARIANT OF PTPN22 USING ANTI-CD3 FAB-DECORATED LIPOPLEXES: IN VITRO EFFICACY OF A NOVEL PERSONALIZED IMMUNOTHERAPEUTIC APPROACH FOR TYPE 1 DIABETES

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INTRODUZIONE

Type 1 diabetes (T1D) and thyroiditis, the most common autoimmune endocrinopathies, are actually managed only through substitutive hormonal administration. However, in T1D this administration does not fully replace the body's requirements, remaining associated with acute/chronic complications. This emphasizes the need to identify personalized immunotherapies targeting T-/B-lymphocytes.The C1858T variant in protein tyrosine phosphatase non-receptor type N22 (PTPN22) gene, encoding R620W Lyp protein, affects both innate/adaptive immunity, leading to a gain-of-function with paradoxical reduced T-cell activation. We developed a novel personalized liposomal-siRNA-based immunotherapy (lipoplexes), that selectively inhibits the variant allele (USA Patent/2025.09.09 n.12.410.431; PCT/IT2019/050095). To improve their delivery to T-lymphocytes, lipoplexes were functionalized with Fab of anti-CD3 MoAb. We aimed to tune the Fab concentration on lipoplexes to warrant efficient PBMC binding without causing activation-induced apoptosis and develop a functional in vitro assay to assess anti-CD3Fab-LiposiRNA efficacy on IL-2 secretion.

METODI

FACS confirmed selective anti-CD3Fab-LiposiRNA binding to Jurkat T-cells and Healthy Donors' PBMC compared to non-functionalized lipoplexes. We estimated the optimal anti-CD3Fab binding-concentration not causing activation-induced apoptosis. In vitro functional efficacy of anti-CD3Fab-LiposiRNA was assessed in C1858T-PTPN22-patients' PBMC by supernatants IL-2 ELISA detection, after lipoplexes preincubation (6 hours) followed by anti-CD3/CD28-beads stimulation. Statistical significance vs wild-type T1D patients was evaluated by one-way ANOVA and Sidàk-Test ($p<0.05$). Confocal microscopy tracked lipoplexes internalization using AlexaFluor488-Fab and ATTO594-siRNA.

RISULTATI

The optimal anti-CD3-Fab concentration on lipoplexes was estimated at 0.05µg/mL. Preincubation of PBMC from C1858T-PTPN22-patients with anti-CD3Fab-LiposiRNA restored IL-2 secretion after anti-CD3/CD28 stimulation, rescuing the gain-of-function-associated impairment on T-cell activation ($p<0.0001$). No increase occurred in wild-type PBMC ($p>0.05$). CM demonstrated efficient lipoplexes internalization after 30 minutes, where Fab and siRNA were separately visualized, avoiding aggregations/masking effects.

CONCLUSIONI

Collectively, these data demonstrate efficacy/tolerability of anti-CD3Fab-LiposiRNA in halting variant effect on T1D PBMC, supporting their translational potential for in vivo evaluation.

B056

NETWORK-BASED APPROACH IDENTIFIES MIR-145-3P AS A CENTRAL REGULATORY HUB ASSOCIATED TO THE PROGRESSION FROM LOCALIZED TO METASTATIC MEDULLARY THYROID CARCINOMA.

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INTRODUZIONE

Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor originating from calcitonin-producing C-cells and accounts for 1–5% of thyroid cancers. Total thyroidectomy is curative in localized disease (N0), whereas lymph node metastases (N1) are associated with poorer prognosis. However, the molecular mechanisms driving the metastatic shift remain poorly understood.
This study aimed to identify miRNA features linked to metastatic spread in MTC, focusing on the transition from N0 to N1.

METODI

MiRNA expression profiles from patient-derived MTC tumors with (N1) or without (N0) lymph node metastases were used to construct co-expression networks. Co-expression networks were constructed for N0 and N1 tumors, and differential connectivity analysis was used to identify key miRNAs acting as regulatory hubs. Functional annotation of their target genes was performed using KEGG, GO, and Reactome pathway analyses. Validation experiments were carried out in MTC cells to evaluate the effects of selected miRNAs on cell proliferation, survival, and MAPK pathway activation.

RISULTATI

Analysis of MTC tumor samples revealed distinct miRNA co-expression networks between N0 and N1 tumors. Differential network analysis identified miR-145-3p as a central regulatory hub, showing 29 altered co-expression relationships and a marked loss of connectivity in N1 tumors. Functional enrichment analysis revealed 59 validated target genes, including major oncogenic regulators such as MYC, PTEN, BCL2, PIK3CA, AKT1, and MAPK7. In MTC cells, simultaneous inhibition of miR-145-3p and its top co-expressed miRNAs promoted cell proliferation and survival and increased ERK phosphorylation, indicating activation of the MAPK signaling pathway and acquisition of a more aggressive phenotype.

CONCLUSIONI

miR-145-3p plays a central role in the regulatory network associated with metastatic progression in MTC. Its loss of connectivity in N1 tumors and the effects observed following its inhibition suggest that disruption of this miRNA network contributes to tumor aggressiveness and metastatic potential. These findings highlight miR-145-3p as a potential biomarker and therapeutic target in metastatic MTC.

B057

IDENTIFICATION OF CIRCULATING TUMOR CELLS (CTCS) IN METASTATIC COLORECTAL CANCER

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INTRODUZIONE

Circulating tumor cells (CTCs) are minimally invasive biomarkers with prognostic relevance in metastatic colorectal cancer. Their identification and enumeration may provide clinically useful information to support prognostic assessment, therapeutic decision-making, and longitudinal monitoring of disease evolution.

METODI

Blood samples were collected in CellSave tubes and processed with the CELLTRACKS AUTOPREP using the CELLSEARCH CTC Kit. Briefly, 7.5 mL of blood underwent EpCAM-based magnetic enrichment with ferrofluid nanoparticles. Enriched cells were stained with anti-cytokeratin-PE and DAPI to identify epithelial nucleated cells, while anti-CD45-APC excluded leukocytes. Cartridges were analyzed with the CELLTRACKS ANALYZER II. Results were interpreted using the mCRC cut-off of >=3 CTCs/7.5 mL.

RISULTATI

The dataset comprised 66 blood-sampling records from metastatic colon cancer patients. These records did not represent 66 independent patients: some were assessed only once, whereas others underwent serial follow-up sampling. Overall, 61/66 samples (92.4%) were evaluable and 5/66 (7.6%) were excluded for pre-analytical or technical reasons. Among evaluable cases, 13 patients had at least two evaluable time points and were suitable for longitudinal CTC monitoring, while the remaining cases contributed single-time-point determinations. At sample level, 26/61 (42.6%) showed at least one CTC, 10/61 (16.4%) were at or above the mCRC cut-off, and 3/61 (4.9%) showed >=5 CTCs/7.5 mL; counts ranged from 0 to 15. At patient level, 43 unique evaluable patients were included: 24/43 (55.8%) had at least one CTC-positive sample and 10/43 (23.3%) reached the cut-off at least once. In the longitudinal subgroup, 6/13 patients showed increased CTC counts from first to last draw, 4/13 decreased and 3/13 remained stable; three patients crossed the >=3 CTCs/7.5 mL cut-off. Ongoing monitoring is concordant with clinical data and disease evolution.

CONCLUSIONI

This preliminary experience supports the feasibility of CELLSEARCH-based CTC detection and longitudinal monitoring in metastatic colon cancer. CTC enumeration may help clinicians assess prognostic and therapeutic trends over time, supporting conventional clinical, laboratory and imaging-based evaluation.

B058

NATURAL KILLER CELLS SUBSETS ALTERATIONS IN MULTIPLE SCLEROSIS: HIGHLIGHTING THE LINK WITH EBV INFECTION AND THE EFFECTS OF DISEASE MODIFYING THERAPY (DMT).

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INTRODUZIONE

Multiple Sclerosis (MS) is a chronic immune-mediated neuroinflammatory disorder affecting the Central Nervous System. Its pathogenesis is characterized by immune dysregulation, focusing on the adaptive immunity, and environmental triggers among which Epstein-Barr Virus (EBV) infection seems to play a crucial role to cause the disease onset. Since EBV control is mediated by NK cells we focused on innate immunity to better comprehend its role in MS and we observed the effect of DMTs on the NK cell compartment.

METODI

MS patients (n=20) were enrolled at disease onset together with Healthy Controls (HC,n=10). PBMCs were collected for each patient and immunophenotype was performed. Moreover, a transcriptomic analysis of isolated NK cells from MS patients (n=10) and HC (n=3) identified a disease specific signature that was FACS-confirmed.

RISULTATI

Immunophenotype analysis showed a significant expansion of CD56brightNK cells in MS patients, while CD56dimNK cells are markedly reduced compared to HC. Consequently, the peripheral CD56bright/CD56dim ratio is substantially increased in MS patients. This altered ratio correlates significantly with EBV-IgG titers, suggesting a potential mechanistic link between viral immunity and NK cell dysregulation in MS pathogenesis. We also observed a significantly elevated the percentage of CD56dimNK cells positive for senescence-associated β-galactosidase(β-gal). Accordingly, also DNA damage marker phosphorylated histone H2AX(γ-H2AX) and Annexin V in CD56dimNK cells MS patients are found higher than HC, indicating accelerated cellular aging and genomic instability within this subset. The transcriptomic analysis on NK cells showed that the differential expression between MS patients and HC was linked to Stimulator of Interferon Genes (STING) pathway, well known to be involved in the immune response in case of viral infection. FACS analysis confirmed that the STING/TBK1/IRF3/INF type I pathway are defective in MS patients when compared to HC. Finally, following DMTs, MS patients had a significant reduction in the percentage of β-gal-positive CD56dimNK cells, of the levels of CD56dimNK cells apoptosis and the restoration of p-TBK1 levels in MS patients, suggesting that a rescue of NK cell homeostasis may contribute to its clinical efficacy.

CONCLUSIONI

These findings reveal the role of NK cells in MS immunopathology and a putative link with EBV infection. Further studies are needed to better understand the role of STING pathway, as a new therapeutic target for disease treatment.

B059

P53 REACTIVATION: A POTENTIAL IMMUNOTHERAPEUTIC STRATEGY TO ENHANCE ANTI-TUMOR IMMUNE RESPONSES IN PAPILLARY THYROID CANCER

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INTRODUZIONE

Cancer evades immune surveillance through multiple strategies, including suppression of effector T (Teff) and increased activity of T regulatory cells (Treg) promoting disease progression. Nowadays, immunotherapeutic approaches using monoclonal antibodies to checkpoint inhibitors have proven efficacy, but their clinical use is limited by autoimmune adverse effects, highlighting the need for improved strategies. As regard, p53 represents a promising therapeutic target, acting both as oncosuppressor and immune regulator. We previously demonstrated that p53 reactivation using an inhibitor of MDM2/MDM4 heterodimerization (Pep3) in PBMCs of pediatric thyroid cancer patients enhances Teff, reduces Treg responses, without affecting activated T cells, thus promoting anti-tumor immunity without autoimmunity progression. The immunoregulatory and transcriptional mechanisms by which Pep3 shapes anti-tumor immunity remain unclear.

We aimed to: 1) investigate the effects of p53 reactivation on NK cells in PBMCs of patients with wild-type p53 papillary thyroid carcinoma, eventually associated with thyroid autoimmunity; 2) unravel p53 reactivation molecular mechanisms underlying tumor-immune crosstalk.

METODI

NK cell degranulation of PBMCs of healthy donors (HD) and patients was evaluated in co-colture with target K562 cell line. Expression of activating/inhibitory ligands recognized by NK receptors in tumor (TPC1) and normal epithelial thyroid (Nthy-ori) cell lines upon Pep3 reactivation was evaluated by qRT-PCR. mRNA levels of immunomodulatory genes in PBMCs upon Pep3 exposure were measured by qRT-PCR. Protein levels of selected immunomodulators in PBMCs upon p53 reactivation were quantified by Ella assay.

RISULTATI

NK cell activation was revealed in both HD/patient PBMCs, particularly in the CD16#CD56# subset of patients. Pep3 treatment upregulated mRNA levels of NK activating-receptor ligands in TPC1 compared to Nthy-ori. P53 reactivation increased expression of immune-related genes CSF1, CXCL1, IL-17, IL-10 and downregulated STAT3 and DD1α in 11 patients' PBMCs. Furthermore, Ella confirmed increased IL-10 levels in patients' PBMCs, compared with HD.

CONCLUSIONI

P53 reactivation increases NK activating-receptor ligands suggesting that, further to enhance Teff/Treg ratio, may strengthen NK cell-mediated anti-tumor immunity. In patients' PBMCs, immune-related gene expression is modulated, particularly through chemokine and cytokine mRNA levels, supporting a role in shaping the immune microenvironment.

B060

SELENIUM-BIOFORTIFIED STRAWBERRIES AS A FUNCTIONAL FOOD: EFFICACY ON GLUCOSE HOMEOSTASIS AND LIVER BIOMARKERS IN HEALTHY ADULTS

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INTRODUZIONE

Micronutrient malnutrition, or "hidden hunger," affects over two billion people worldwide and is partly driven by soil nutrient depletion due to intensive agriculture. Agronomic biofortification is a sustainable and cost-effective strategy to enhance the concentration of essential trace elements in foods. Selenium (Se) is an essential micronutrient involved in antioxidant defense and metabolic regulation. This pilot study evaluated the safety and efficacy of daily consumption of Se-biofortified strawberries on glucose homeostasis and liver function in healthy adults, compared with a synthetic Se supplement.

METODI

A 30-day randomized, controlled, single-blind nutritional intervention was conducted in 35 healthy adults (18-65 years) in Palermo, Italy. Participants were assigned to three groups: Control (n=12), consuming 100 g/day of standard strawberries; Se-Strawberry (n=12), consuming 100 g/day of Se-biofortified strawberries (Florida Fortuna; 27 +- 3.3 ug Se/100 g); and Se-Tablet (n=11), receiving 100 ug Se/day as a commercial supplement. At baseline (T0) and after intervention (T1), body composition was assessed by bioelectrical impedance analysis, while fasting blood samples were analyzed for serum Se, glucose, insulin, HOMA indices, and liver biomarkers (AST, ALT, GGT, albumin).

RISULTATI

Agronomic biofortification produced a 4-fold increase in strawberry Se content. A significant rise in blood Se levels was observed only in the Se-Strawberry group (+80%, from 76 +- 20.3 to 132 +- 45.7 ug/L; p=0.0005). This group also showed significant metabolic improvements, including reductions in fasting glucose (-10.6%, p=0.0002), insulin (-36.4%, p=0.0242), HOMA-IR (-38.5%, p=0.0178), and increases in HOMA %S (+69.3%, p=0.002). Liver markers also improved significantly: AST (-24.4%, p=0.01), ALT (-55.6%, p=0.04), and GGT (-46.8%, p=0.002). No significant changes were observed in the control or tablet groups.

CONCLUSIONI

Daily consumption of Se-biofortified strawberries was safe and mildly effective in improving glucose homeostasis and liver biomarkers in healthy adults. The absence of metabolic effects in the synthetic supplement group suggests that the whole food matrix may enhance Se bioavailability through interactions with natural phytochemicals. This approach may support the development of functional foods for the primary prevention of metabolic disorders.

B061

DEPOT-SPECIFIC REGULATION OF ADIPOGENIC AND THERMOGENIC PROGRAMS IN HUMAN ADIPOSE STEM CELLS BY GLP1 RECEPTOR MODULATION

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INTRODUZIONE

Obesity is associated with impaired adipose tissue plasticity and metabolic dysfunction. Glucagon-like peptide-1 receptor agonists (GLP1RAs) are widely used for obesity treatment, but their direct effects on human adipose-derived stem cells (ASCs) remain unclear. This study investigated whether GLP1 receptor (GLP1R) modulation influences adipogenic and thermogenic differentiation of human ASCs.

METODI

Human ASCs isolated from subcutaneous adipose tissue of different overweight donors were characterized and induced toward white adipogenic (WAT) or brown/beige thermogenic (BAT) differentiation. Cells were treated with the GLP1R agonist beinaglutide (BN) or the antagonist Exendin 9-39 (EX939). Cell viability, cell-cycle distribution, lipid accumulation, intracellular cAMP levels, and GLP1R protein expression were assessed. Gene expression of NANOG, C/EBPα, PPARγ, and UCP1 was analyzed by quantitative PCR.

RISULTATI

Both BN and EX939 preserved ASC viability and induced only minor changes in cell-cycle progression. GLP1R modulation significantly suppressed white adipogenic differentiation, as demonstrated by reduced C/EBPα and PPARγ expression. In parallel, UCP1 expression increased, indicating activation of thermogenic programs. Notably, these effects were depot-dependent. In BAT-derived cells, EX939 maintained PPARγ and UCP1 expression, whereas BN reduced BAT-specific markers. In WAT-derived cells, both treatments promoted partial browning, characterized by increased UCP1 expression and modulation of adipogenic transcription factors. Different cAMP responses were also observed, with BN increasing cAMP levels in WAT and decreasing them in BAT, while EX939 exerted opposite effects. Western blot analysis confirmed GLP1R expression in ASCs and revealed differential regulation according to adipose phenotype.

CONCLUSIONI

CONCLUSIONS: GLP1R agonism and antagonism directly influence human adipose progenitor fate by controlling adipogenic pathways and promoting thermogenic features in a depot-specific manner. These findings identify GLP1 signalling as a regulator of adipose tissue plasticity and provide new mechanistic insights into the metabolic benefits of incretin-based therapies.

B062

TARGETING TUMOR CELL MIGRATION IN OSCC: EFFECTS OF COLD ATMOSPHERIC PLASMA (CAP) ON CYTOSKELETON AND ADHESION PATHWAYS

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INTRODUZIONE

Tumor cell migration plays a crucial role in invasion and metastatic progression of oral squamous cell carcinoma (OSCC). Although cold atmospheric plasma (CAP) has become a new anticancer treatment, its effects on cell motility remain largely unexplored. This work investigate the impact of CAP on OSCC cell migration, cytoskeletal regulation and the epithelial–mesenchymal transition (EMT).

METODI

Human head and neck squamous cell carcinoma (HNSCC) cell lines were exposed to CAP under standardized conditions. Cell migratory capacity was quantitatively evaluated using wound healing assays in presence of a DNA synthesis inhibitor. Western blot analysis was carried out to assess the expression of proteins linked to the EMT. Signaling pathways linked to cell migration and cytoskeletal dynamics in HNSCC were identified by bioinformatic analysis.

RISULTATI

CAP treatment significantly impaired migration in OSCC lines, as demonstrated by delayed wound closure compared to controls. Bioinformatic analyses revealed enrichment of pathways involved in actin cytoskeleton regulation, focal adhesion, and cell motility in HNSCC. CAP therapy led to a significant E-cadherin upregulation, unaltered N-cadherin expression, suggesting a transition toward a less invasive phenotype.

CONCLUSIONI

CAP inhibits OSCC cell migration and modulates adhesion-related signaling and cytoskeletal dynamics, suggesting a role in reducing tumor invasiveness. These results improve the evidence for implementing CAP into innovative treatment approaches to reduce tumor growth and metastases in HNSCC.

B063

MODULATION OF SMALL DENSE LDL CHOLESTEROL BY ECKLONIA MAXIMA BIOFORTIFIED VEGETABLES: A TRANSLATIONAL NUTRITIONAL STRATEGY TARGETING CARDIOVASCULAR RISK BIOMARKERS

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INTRODUZIONE

Vascular aging and atherosclerotic progression are driven by complex pathophysiological mechanisms where oxidative stress, endothelial dysfunction, and low-grade chronic inflammation (inflammaging) act synergistically. Small dense low-density lipoprotein cholesterol (sdLDL-C) is a highly atherogenic fraction strongly associated with endothelial dysfunction, insulin resistance, and cardiovascular disease. Identifying interventions capable of modulating sdLDL-C represents a relevant objective in translational medicine, where biomarkers assess disease risk and therapeutic efficacy. This study investigated whether vegetables biofortified with polyphenols and antioxidant-rich Ecklonia maxima extract could influence sdLDL-C levels in healthy adults.

METODI

A 4-weeks, randomized, single-blind, controlled dietary intervention was conducted in 27 healthy volunteers (aged 50-65 years). Participants consumed 100 g/day of biofortified lettuce (Lactuca sativa L) cultivated using Kelpak®, an Ecklonia maxima-derived biostimulant produced through Cold Cellular Burst Technology. At baseline (T0) and post-intervention (T1), next-generation lipid biomarkers (sdLDL), stress-related hormonal profiles (cortisol), and systemic pro-inflammatory cytokines (IL-6 and CRP) were quantified.

RISULTATI

Consumption of biofortified vegetables was associated with a significant reduction in circulating sdLDL-C concentrations compared with baseline values and control conditions. The enhanced antioxidant and polyphenolic content of the biofortified vegetables may contribute to these effects through modulation of oxidative stress and lipid homeostasis. Furthermore, the biofortified group showed favorable shifts in hormonal and inflammatory systemic markers, including a significant reduction in cortisol, interleukin-6 and C-reactive protein alongside a significant increase of Vitamin D.

CONCLUSIONI

These findings identify dietary biofortification with Ecklonia maxima as an effective strategy for modulating sdLDL-C, a clinically relevant biomarker of cardiovascular risk. The study supports the integration of nutritional interventions within a translational medicine framework, linking food innovation to biomarker-guided prevention of chronic cardiometabolic disease.

B064

DECIPHERING RNA-DEPENDENT ERα CHROMATIN AND TRANSCRIPTIONAL REGULATION TO IDENTIFY NOVEL THERAPEUTIC VULNERABILITIES IN BREAST CANCER

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INTRODUZIONE

Endocrine resistance remains a major challenge in estrogen receptor alpha (ERα)-positive breast cancers (BCs), driven by adaptive rewiring of transcriptional and chromatin regulatory networks. Beyond its canonical transcriptional activity, ERα also functions as an RNA-binding protein (RBP), interacting with long non-coding RNAs (lncRNAs) involved in regulatory plasticity. Among these, PVT1 has emerged as an oncogenic ERα-interacting lncRNA implicated in transcriptional regulation. This study investigated the role of RNA-mediated ERα activity and the epigenetic scaffold PVT1 in shaping chromatin architecture and transcriptional programs associated with BC progression.

METODI

PVT1 was silenced in ERα-positive MCF-7 BC cell models using antisense oligonucleotides (ASOs). Genome-wide chromatin organization was assessed through high-throughput chromosome conformation capture (Hi-C). In addition, Hi-C analyses were also performed in MCF-7 clones carrying mutations in the ERα RNA-binding domain (RBD). Transcriptome profiling by bulk RNA-seq was conducted to identify differentially expressed genes and shared regulatory signatures associated with chromatin alterations.

RISULTATI

Hi-C analyses revealed highly similar chromatin organization patterns in PVT1-depleted cells and ERα RNA-binding-deficient clones, supporting a functional interplay between PVT1 and the RNA-binding activity of ERα. Both conditions displayed comparable compartmentalization signatures and coordinated alterations in higher-order genome organization, associated with shared transcriptional changes involving tumor-related genes. These findings indicate that disruption of the PVT1–ERα regulatory axis similarly affects chromatin dynamics and transcriptional outputs linked to BC progression.

CONCLUSIONI

Our results identify PVT1 and ERα RNA-binding activity as key regulators of chromatin plasticity and transcriptional regulation in ERα-positive BCs. Targeting RNA-mediated ERα regulatory mechanisms may represent a novel therapeutic strategy to interfere with estrogen signaling, limit tumor evolution, and overcome endocrine resistance.

B065

A COMPOSITE INFLAMMATORY-RENAL PROGNOSTIC SCORE PREDICTS IN-HOSPITAL MORTALITY IN OLDER ADULTS WITH SEPSIS

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INTRODUZIONE

Sepsis remains a leading cause of mortality in older hospitalised patients. Identifying readily available prognostic biomarkers is critical for risk stratification. The neutrophil-to-lymphocyte ratio (NLR) and the C-reactive protein-to-albumin ratio (CAR) are inexpensive inflammatory indices, yet their combined predictive value in older patients with sepsis is poorly characterized. This study aimed to evaluate the association of NLR, CAR, and estimated glomerular filtration rate (eGFR) with in-hospital mortality, and to derive a composite scoring tool, termed the NCe score (NLR-CAR-eGFR).

METODI

A retrospective cohort study from the Report-AGE project was conducted on 846 patients aged ≥75 years admitted at the IRCCS INRCA hospital with sepsis or septic shock. NLR, CAR, and eGFR were measured at admission. Cox proportional hazards regression models were fitted, adjusted for age, sex, transaminases, RDW-CV, Charlson Comorbidity Index (CCI), polypharmacy and functional impairment.

RISULTATI

In-hospital mortality occurred in 258 patients (30.5%). Deceased patients had significantly higher NLR (12.5 vs 9.0, $p<0.001$), higher CAR (0.5 vs 0.4, $p=0.024$), and lower eGFR (34.2 vs 42.6 mL/min/1.73m², $p<0.001$). On multivariable Cox analysis, the highest NLR tertile (HR 1.57, 95%CI 1.12-2.20, $p=0.009$) and the intermediate CAR tertile (HR 1.44, 95%CI 1.05-2.01, $p=0.023$) were independently associated with mortality. The composite NCe score (combining NLR tertile, CAR tertile and eGFR category) showed a stepwise increase in mortality risk (HR 2.12, 95%CI 1.41-3.19, $p<0.001$).

CONCLUSIONI

NLR, CAR and eGFR are independent predictors of mortality in older patients with sepsis. Their combination in the NCe score provides a simple, widely accessible tool for bedside risk stratification.

B066

KCASH2-TARGETING MICRORNAS IN COLORECTAL CANCER: FROM IN VITRO MODELS TO CANDIDATE CIRCULATING BIOMARKERS

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INTRODUZIONE

Aberrant activation of the Sonic-Hedgehog (Hh) pathway is implicated in several tumors, including colorectal cancer (CRC). Although its role in CRC remains unclear, Gli, the final Hh effectors, may contribute to early CRC carcinogenesis. Histone deacetylase HDAC1 enhances Hh signaling by deacetylating Gli1/2, whereas the KCASH proteins act as tumor suppressors by promoting HDAC1 ubiquitination and degradation, thereby inhibiting Hh-driven tumor cell proliferation. Notably, KCASH2 is described as down-regulated in CRC. This study aimed to identify miRNAs specifically targeting KCASH2 and evaluate their potential as candidate biomarkers in CRC.

METODI

TaqMan-based miRNA profiling was performed in five CRC cell lines, showing different KCASH2, Gli1 and HDAC1 protein expression levels. In silico analyses (Tarbase, microT-CDS, Targetscan, miRDB) were performed to identify miRNAs targeting KCASH2. Public datasets (GEO, dbDEMC) were interrogated to assess levels of the most interesting miRNAs in serum/plasma of CRC patients compared to healthy subjects. Validation was carried out by TaqMan qRT-PCR in plasma from CRC patients, collected before and after surgical tumor resection, and from healthy individuals.

RISULTATI

MiRNA profiling revealed several differentially expressed miRNAs. Integrative analysis identified 18 miRNAs targeting KCASH2. Among them, miR-24-3p, miR-196b-5p, and miR-27-3p emerged as the most promising candidate, being already described in CRC and classified as oncomiRs. Analysis of public circulating miRNA datasets consistently showed upregulation of these miRNAs in CRC patient plasma. Validation revealed significantly increased plasma levels of the three miRNAs in pre-surgery samples from CRC patients compared to healthy subjects. Comparing pre- and post-surgery plasma samples revealed heterogeneous expression patterns, likely reflecting differences in clinicopathological and molecular tumor features.

CONCLUSIONI

MiRNA profiling of CRC cells with different KCASH2 expression identified miR-24-3p, miR-196b-5p,and miR-27-3p which were also significantly upregulated in plasma from CRC patients. These findings provide new insights into the epigenetic regulation of Hh pathway in CRC, thus evidencing new candidate biomarkers for KCASH/Hh-related CRC patients.

B067

TARGETING THE MMR SYSTEM TO SENSITIZE CHEMORESISTANT MICROSATELLITE STABLE GASTRIC CANCER TO IMMUNOTHERAPY

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INTRODUZIONE

Gastric cancer (GC) is a major global health burden with high mortality. While microsatellite instability-high (MSI-H) tumors benefit from immune checkpoint inhibitors, the vast majority of GC is microsatellite stable (MSS), retaining a functional mismatch repair (MMR) system associated with therapy resistance. The MMR complex plays a key role in maintaining genomic integrity; genistein, a soy-derived isoflavone, is a potent modulator of DNA damage responses, potentially capable of interfering with MMR activity. Hypothesising that genistein-induced MMR inhibition can lead to genomic destabilization and reprogram chemoresistant MSS GC tumors toward an immunogenic "MSI-like" phenotype, this project aims to dissect the role of the MMR pathway in GC chemoresistance, and evaluate genistein's capacity to drive this phenotypic reprogramming toward immunogenicity.

METODI

Wild-type (WT) and chemoresistant (FLOTr) MSS AGS and MSI ACC-201 GC cell viability was assessed following genistein treatment via MTT and LIVE/DEAD assays. MMR gene and protein expression were quantified by qPCR and Western blot (WB). Genistein-treated GC cells were co-cultured with PBMCs, and qPCR was performed to quantify the expression of immune-related markers.

RISULTATI

Genistein treatment induced a dose-dependent increase in cell death across both MSS and MSI cells. A more pronounced cytotoxicity was observed in WT AGS cells compared to FLOTr, supporting that FLOTr cells upregulate survival-associated MMR factors. Baseline qPCR and WB confirmed that MMR was overexpressed in FLOTr cells. Crucially, genistein treatment successfully downregulated MMR expression in both WT and FLOTr cells, highlighting its role as an inducer of genomic instability. Consequently, co-culturing treated cells with PBMCs stimulated a significant upregulation of cytotoxicity-related markers within the PBMC population.

CONCLUSIONI

This study demonstrates that FLOTr GC cells upregulate the MMR pathway, which can be reversed by genistein to drive an "MSI-like" phenotype in MSS tumors. By inhibiting the MMR system, genistein induces chemosensitivity and immunogenicity in such resistant cells. Overall, this study prepares the way for innovative therapeutic strategies combining genistein with immunotherapy to overcome chemoresistance in MSS gastric cancer.

B068

NEUROGLOBIN REDUCES STRESS-INDUCED OXIDATIVE DAMAGE AND AMYLOID BURDEN IN A THREE-DIMENSIONAL HUMAN NEUROBLASTOMA CELL CULTURE

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INTRODUZIONE

Neuroglobin (Ngb) is a hemeprotein expressed in neurons that acts as a neuroprotectant against multiple brain injuries, including hypoxia, oxidative stress, and β-amyloid toxicity. In addition to inhibiting apoptosis and scavenging ROS/RNS, Ngb stimulates autophagy, which helps preserve cellular homeostasis under basal and stress conditions. Although Ngb upregulation is effective in several animal models of neural disease, its biological behavior has never been studied in three-dimensional (3D) cell cultures, which better mimic in vivo tissue architecture than monolayer cultures. Thus, we developed a 3D model of human SH-SY5Y neuroblastoma cells overexpressing Ngb to test their response to 1-methyl-4-phenylpyridinium (MPP+), a well-established in vitro model of Parkinson's disease-related neurodegeneration.

METODI

Vector-transfected (CTRL) and Ngb-overexpressing (NGB-FLAG) spheroids were obtained by the hanging drop method. Ngb expression was preliminarily tested by Western blot (WB) and confocal microscopy. MPP+-induced oxidative stress and apoptosis were evaluated by flow cytometry (FC) and PARP immunoblot. Additionally, MPP+-induced autophagy impairment and amyloid burden were assessed by WB analysis of detergent-soluble and insoluble LC3-II and p62, and by Thioflavin-S staining and FC analysis, respectively.

RISULTATI

Ngb overexpression reduced MPP+-induced ROS production and cell death, as shown by decreased DCF MFI, percentage of hypodiploid cells, and cleaved PARP-1 levels in MPP+-treated NGB-FLAG spheroids compared to CTRL. Intriguingly, through biochemical fractionation and amyloid detection by Thioflavin-S, Ngb upregulation was shown to decrease MPP+-induced autophagy impairment and amyloid burden, known pathogenic hallmarks of neurodegenerative disorders.

CONCLUSIONI

Altogether, our findings support a role for Ngb as a regulator of neuronal proteostasis and intracellular trafficking under both physiological and pathological conditions. These preliminary findings pave the way for the use of 3D cell cultures to gain further insight into the biological functions of Ngb in health and disease, potentially improving the translational relevance of in vitro neurodegeneration models.

B069

DISSECTING IMPORTIN ALPHA ISOFORMS IN ERK5 NUCLEAR TRANSLOCATION: IMPLICATIONS FOR TARGETED THERAPY IN MELANOMA

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INTRODUZIONE

ERK5 is a promising therapeutic target in cancer whose pro-proliferative activity depends on its nuclear localization. Due to its high molecular weight, ERK5 requires active nuclear transport mediated by heterodimeric importins through the C-terminal classical nuclear localization signal (cNLS). Having recently identified importin ß1 as a key mediator of this process, this study aims to identify the specific importin α isoforms (KPNA1-7) involved in ERK5 nuclear translocation.

METODI

TCGA melanoma datasets were analyzed using TNMplot and cBioPortal to assess the expression of importin α isoforms in tumor vs normal tissues and their correlation with ERK5 target genes. siRNA-mediated knockdown (KD) of importin α isoforms was performed to evaluate their effect on cell proliferation and ERK5 nuclear translocation by confocal immunofluorescence in unstimulated A375 melanoma cells and EGF-stimulated HeLa cells. Co-immunoprecipitation experiments were carried out to assess the physical interaction between ERK5 and importin α isoforms in HeLa cells expressing basal ERK5 levels and transiently transfected with the constitutively active form of MEK5 (MEK5DD).

RISULTATI

In silico analysis using TNMplot revealed significant upregulation of KPNA1-4 in melanoma, with KPNA2 being the most overexpressed in both primary and metastatic tumors. Both KPNA2 and ERK5 expression positively correlated with proliferation-associated genes, including Cyclin D1, PCNA, Ki67. Accordingly, KPNA2 KD reduced A375 melanoma cell proliferation, whereas silencing of the other isoforms had no significant effect. Co-immunoprecipitation experiments revealed that ERK5 physically interacts with KPNA2 in HeLa cells. Consistently, immunofluorescence analysis showed that both KPNA1 and KPNA2 silencing significantly reduce nuclear ERK5 in A375 and EGF-stimulated HeLa cells. Furthermore, cBioPortal provided evidence of a positive correlation between KPNA2 and the ERK5 target genes c-MYC and MEF2D.

CONCLUSIONI

KPNA2 plays a dual role in melanoma by promoting cell proliferation and mediating ERK5 nuclear translocation. Targeting the ERK5-KPNA2 interaction represents a potential therapeutic strategy to inhibit ERK5 nuclear import and its oncogenic transcriptional activity in melanoma.

B070

BLOCKADE OF PD-L1 ENDOCYTOSIS BY UPAR ANTAGONIST PEPTIDES TO IMPROVE CANCER IMMUNOTHERAPY

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INTRODUZIONE

Immune Checkpoint Inhibitors (ICIs) have revolutionized clinical outcomes in malignancies such as Non-Small-Cell Lung Cancer (NSCLC), particularly in patients with high PD-L1 expression. Recent evidence shows that targeting extracellular Plasminogen Activator Inhibitor (PAI-1) synergizes with anti-PD-L1 blockade in melanoma. PAI-1, a serpin superfamily member, inhibits tissue (tPA) and urokinase-type (uPA) plasminogen activators. Mechanistically, PAI-1 binds the uPA/uPAR complex, recruiting LRP-1 and triggering endocytosis of the PAI-1-uPA-uPAR-LRP-1 quaternary complex. This process induces the internalization and lysosomal degradation of surface PD-L1. Since many aggressive tumors overexpress uPAR, inhibiting uPAR to prevent PD-L1 endocytosis—thereby maintaining high cell-surface levels—could enhance anti-PD-1/PD-L1 immunotherapy in NSCLC and pancreatic cancer models.

METODI

A549 (NSCLC) and BxPC-3 (pancreatic cancer) cells, characterized by baseline PD-L1 expression, were grown in 2D and 3D cultures and treated with the uPAR antagonist peptide IPR803 (2μM). PD-L1 membrane expression was evaluated via FACS, confocal microscopy, and western blotting. To assess T-cell-mediated cytotoxicity, 3D co-cultures of tumor cells (pre-treated with anti-human PD-L1 and IPR803) and CD8+/CD4+ T cells were analyzed using Live/Dead staining. Additionally, patient-derived organoids (PDOs) were isolated from pleural effusions of NSCLC patients, treated with IPR803 2μM, and analyzed for surface PD-L1 expression by FACS and confocal immunofluorescence microscopy.

RISULTATI

In 2D and 3D cultures of A549 and BxPC-3, IPR803 successfully blocked PD-L1 internalization, significantly increasing its surface expression. In 3D co-culture assays, the combined treatment of IPR803 and anti-PD-L1 antibody markedly enhanced T-cell cytotoxic activity against tumor cells. Furthermore, brightfield microscopy confirmed the feasibility of culturing primary PDOs from patient pleural effusions. Confocal microscopy validated that 2μM IPR803 treatment induces a significant increase in PD-L1 membrane expression also in PDOs.

CONCLUSIONI

Our findings demonstrate that uPAR inhibition via the antagonist peptide IPR803 stabilizes PD-L1 at the cell surface by preventing its endocytosis. This mechanism significantly enhances T-cell-mediated cytotoxicity, supporting the rationale for novel combined therapeutic strategies pairing uPAR inhibitors with anti-PD-1/PD-L1 ICIs.

B071

THE CYTOSKELETAL FORMINS INF2 AND MDIA DIFFERENTIALLY REGULATE THE SONIC HEDGEHOG PATHWAY AND MEDULLOBLASTOMA TUMORIGENESIS

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INTRODUZIONE

Sonic Hedgehog medulloblastoma (SHH-MB) is a pediatric cerebellar tumor driven by aberrant SHH pathway activation. While several regulators of SHH signaling have been identified, the mechanisms sustaining SHH-MB growth remain poorly understood. Emerging evidence suggests that cytoskeletal remodeling plays an active role in cancer progression, yet its contribution to SHH signaling and SHH-MB biology is largely unexplored. Here, we investigate the role of INF2 and mDia formins, key regulators of cytoskeletal dynamics, in SHH signaling and SHH-MB tumorigenesis.

METODI

SHH pathway activity was evaluated by GLI1 expression and transcriptional assays following INF2 and mDia overexpression or silencing. Biological assays were performed in granule cell progenitors (GCPs) and SHH-MB primary cells, after formins expression modulation. INF2 mutants defective in mDia binding were generated to dissect the mechanisms underlying INF2-mediated regulation of SHH signaling.

RISULTATI

We identified INF2 as a negative regulator of SHH signaling, acting in opposition to mDia formin, shown to promote SHH pathway activation. INF2 overexpression counteracts the positive effects of mDia on GLI1, the final SHH effector, by reducing its transcriptional activity and expression. We demonstrated that the repressive effect of INF2 on SHH signaling is directly linked to its interaction with mDia. Indeed, INF2 mutants unable to bind mDia fail to decrease GLI1 expression and activity. Moreover, we found that INF2 expression rises as SHH signaling declines during normal cerebellar development in mice, showing an inverse trend to mDia. Accordingly, INF2 silencing increases GLI1 expression and promotes the proliferation of GCPs, the cells of origin of MB. Notably, INF2 protein levels are strongly reduced in SHH-MBs, while mDia is upregulated. INF2 overexpression or mDia silencing significantly inhibited SHH-MB proliferation, while INF2 mutants defective in mDia binding lost this inhibitory effect.

CONCLUSIONI

Our findings identify INF2 and mDia as opposite regulators of SHH signaling and SHH-MB growth. The INF2/mDia interaction emerges as a critical molecular node controlling GLI1 and tumor proliferation, highlighting the INF2/mDia axis as an innovative therapeutic target in SHH-MB.

B072

PRO-ANGIOGENIC RECEPTOR SHEDDING DRIVES AN ANGIOSTATIC SWITCH IN CHRONIC HYPOXIC GASTROINTESTINAL CANCERS

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INTRODUZIONE

Gastrointestinal cancers (GIC) are high-stromal tumors that severely impair functional vasculature. This dense stroma exacerbates intratumoral hypoxia, a critical driver of malignancy, metabolic reprogramming, and chemoresistance. While acute hypoxia triggers robust angiogenic responses, the effects of chronic hypoxia within this dense microenvironment remain complex. This study investigates the molecular shift occurring during prolonged oxygen deprivation, focusing on how chronic hypoxic conditions alter angiogenic signaling through the generation of decoy receptors.

METODI

Nine human GIC cell lines were cultured under normoxic, acute hypoxic (0.5% O_2 for 24 hours), and chronic hypoxic (0.5% O_2 for 72 hours) conditions. Pro- and anti-angiogenic factors in conditioned media were characterized using high-throughput protein arrays, while matrix metalloproteinase (MMP) activity was quantified via gelatin zymography. The functional impact of chronic hypoxic secretome on endothelial cell behavior was evaluated using a membrane-free 3D microfluidic platform for real-time angiogenesis sprouting and endothelial barrier integrity assays.

RISULTATI

High-throughput arrays confirmed a profound divergence in shedding kinetics across the GIC panel. A subset of lines exhibited an immediate proteolysis phase marked by a transient spike in MMPs and soluble receptors under acute hypoxia. Conversely, prolonged chronic hypoxia triggered a delayed, massive accumulation of soluble decoy receptors (such as sVEGFR2, sVEGFR3, and suPAR) mainly in colon but also across several gastric and pancreatic models. Tested on the microfluidic platform, this decoy-laden, protease-rich chronic secretome completely failed to induce vessel sprouting and caused a dramatic loss of endothelial barrier integrity. By sequestering free growth factors, these shed receptors neutralized pro-angiogenic signaling, driving an angiostatic phenotype.

CONCLUSIONI

These findings reveal a novel feedback loop where chronic hypoxia, via MMP- or uPAR-mediated receptor shedding, shifts the microenvironment from pro-angiogenic to angiostatic. Crucially, while angiogenic receptors are actively transcribed, their proteolytic cleavage completely inverts their functional role, highlighting the indispensable need for functional proteomic characterization in stroma-dense tumors, as solely transcriptomic approaches might be scientifically misleading in this context.

B073

INTERPRETABLE MULTI-OMICS MACHINE LEARNING MODEL FOR BREAST CANCER CLASSIFICATION AND BIOLOGICAL CHARACTERIZATION

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INTRODUZIONE

Breast Cancer (BC) is a highly heterogeneous disease characterized by complex genomic and epigenomic alterations. Large-scale studies such as The Cancer Genome Atlas have shown that breast tumors are defined not only by gene-level mutations, but also by chromosomal aberrations and epigenetic dysregulation, including recurrent copy number alterations (CNAs) and global DNA methylation changes. In particular, arm-level CNAs reflect broad genomic instability patterns associated with tumor subtype and tissue identity [1]. In this context, machine learning (ML) approaches, have emerged as effective tools for integrating multi-omic data and identifying molecular determinants of cancer classification. This study aimed at developing an interpretable ML model able to distinguish BC from other tumor types using genomic and epigenomic features.

METODI

Multi-omic data from 8256 samples spanning 32 different cancer types were obtained from the TCGA PanCancer Atlas project. Somatic point mutation burden, CNAs and DNA methylation levels at chromosome arm-level, were considered as predictive features. A stacking classifier, combining XGBoost and SVM models, was developed to classify BC versus other tumor types. Model interpretability was assessed using the SHAP framework, enabling both global and sample-level evaluation of feature contributions.

RISULTATI

The classifier achieved excellent predictive performance, with balanced accuracy of 0.96 and F1-score of 0.98. DNA methylation features were the main drivers of model predictions, showing consistently higher SHAP contributions across chromosomal arms. CNAs contributed to a lesser but complementary extent, while mutation-based features displayed limited importance. Chromosomal arms 8p, 1q, 2q, and 19q emerged as key predictive regions. Among these, 8p methylation levels showed the strongest impact, whereas 1q exhibited a more complex multi-layered contribution involving methylation, CNAs and mutations.

CONCLUSIONI

Using low-dimensional multi-omic features, we developed a robust and interpretable pan-cancer classifier capable of accurately distinguishing BC from other tumor types, also highlighting genomic and epigenomic determinants underlying tissue-specific identity and BC-associated alterations.

B074

NON-CANONICAL START CODON SELECTION OF MYC DRIVES METASTASIS IN COLORECTAL CANCER

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INTRODUZIONE

Metastasis represents the primary cause of mortality in colorectal cancer (CRC) patients. While the Epithelial-Mesenchymal Transition (EMT) facilitates invasion, may paradoxically impair distant colonization, highlighting a need to understand the molecular mechanisms balancing these states. The MYC proto-oncogene is a key regulator of CRC, expressed as a canonical isoform (MycS, p64) and an N-terminally extended isoform (MycL, p67) originating from an alternative CTG start site. We identified a regulatory region in the MYC 5'UTR, termed the Upstream Region (UR), which is essential for regulating alternative start-codon usage rather than cap-independent translation.

METODI

We used CRISPR-Cas9 to generate UR deletion (ΔUR) and selective MycL-knockout (ΔMycL) CRC cell models. Molecular and phenotypic consequences were assessed via RNA-Seq, immunoblotting, migration assays, and metabolic profiling (Seahorse OCR/ECAR). We evaluated metabolic responsiveness using nutrient starvation-recovery assays (specifically Leucine and Methionine) and analyzed metastatic behavior in vivo through intraperitoneal and tail-vein injection models monitored by bioluminescence.

RISULTATI

The UR region functions as a molecular rheostat for MYC start-codon selection. ΔUR and ΔMycL cells exhibit a convergent phenotype: upregulation of EMT markers, increased migration/invasion in vitro, and a profound metabolic shutdown (reduced OCR and ECAR), indicative of a dormant mesenchymal state. Exogenous MycL reintroduction successfully rescued these phenotypes. We demonstrated that Methionine availability serves as a critical substrate, triggering a UR-dependent translational switch from the canonical ATG to the non-canonical CTG site. In vivo, ΔUR and ΔMycL models displayed a drastic reduction in metastatic colonization capacity, confirming that MycL depletion "locks" cells in a mesenchymal state that promotes local invasion but precludes metastatic engraftment.

CONCLUSIONI

These findings reveal a novel axis where Methionine availability controls alternative MYC start-codon selection via the UR region. MycL acts as a rheostat governing metastatic fitness: its depletion induces a quiescent mesenchymal state that facilitates local invasion while preventing the metastatic colonization. This translational switch represents a promising therapeutic target to selectively inhibit the colonization phase of CRC metastasis.

B075

PRECLINICAL EVALUATION OF THE TISSUE SELECTIVITY OF NOVEL THRβ AGONISTS

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INTRODUZIONE

The triiodothyronine/Thyroid Hormone Receptor beta (T3/THRβ) axis is frequently downregulated in high-grade hepatocellular carcinoma (HCC) and during early hepatocarcinogenesis. Intracellular hypothyroidism is also associated with metabolic dysfunction-associated steatotic liver disease (MASLD), a major risk factor for HCC. In preclinical models, pharmacological reactivation of THRβ with high-dose T3 induces regression of both MASLD and advanced HCC, supporting the therapeutic potential of restoring thyroid hormone signaling. However, clinical application is limited by THRα-mediated cardiovascular toxicity. This has driven the development of THRβ-selective agonists, including resmetirom, although their efficacy against established HCC appears modest. To address this limitation, the novel halogen-free THRβ agonists TG68 and its active metabolite IS25 were developed and shown to exert anti-steatogenic and antitumorigenic effects in rodents without evident systemic toxicity. We therefore hypothesize that these beneficial effects are THRβ-dependent and can be achieved without significant extrahepatic adverse effects.

METODI

To investigate potential extrahepatic toxicity, ongoing transcriptomic analysis (RNA-seq) of rats treated with T3, IS25, or TG68 aims to characterize tissue-specific molecular responses and assess the hepatic selectivity of the compounds. Transcriptomic data will be integrated with histological and functional evaluations of cardiac and renal tissues to identify liver-specific therapeutic signatures and to assess potential off-target and toxicity-related effects.

RISULTATI

Preliminary analyses indicate a favorable safety profile for the novel thyromimetics, with no histological evidence of cardiac or renal toxicity in chronically treated animals. Ongoing transcriptomic characterization will provide insight into tissue-specific responses to T3, IS25, and TG68, helping define hepatic pathways associated with therapeutic activity, assess potential extrahepatic effects, and further characterize the liver selectivity of these compounds.

CONCLUSIONI

TG68 and IS25 are promising THRβ-selective thyromimetics with anti-steatogenic and anti-neoplastic potential. Preliminary findings suggest a favorable safety profile, and ongoing transcriptomic analyses will further clarify their tissue selectivity and translational potential in MASLD-associated HCC.

B076

TARGETING DDR2 ENHANCES CD28×MUC1 BISPECIFIC ANTIBODY IMMUNOTHERAPY IN SEROUS OVARIAN CARCINOMA

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INTRODUZIONE

Serous ovarian carcinoma (SOC) is a highly aggressive gynaecological malignancy with frequent relapse and the development of chemoresistance. A major feature of SOC tumour microenvironment is the accumulation of dense collagen fibres, which promote tumour progression and immune evasion. Discoidin domain receptor 2 (DDR2), a collagen-binding receptor tyrosine kinase activated by fibrillar collagens, mediates extracellular matrix-associated signalling involved in tumour progression and immune exclusion. However its role in limiting antitumour immunity and contributing to immunotherapy resistance in SOC remains poorly understood.

METODI

We investigated the impact of DDR2 blockade on SOC-specific T cell activity and on the efficacy of recombinant bispecific antibodies (BsAbs) targeting CD28 and mucin 1 (MUC1). SOC-specific T cells were generated from healthy donor peripheral blood mononuclear cells and assessed for recruitment, activation, and cytotoxicity against low-grade (OVCA433) and high-grade (OVCAR3) SOC cell lines under different treatment conditions.

RISULTATI

DDR2 inhibition enhanced SOC-specific T cell responses by remodelling the collagen matrix, favouring their recruitment, activation and cytotoxic activity against both low- and high-grade SOC cells. Moreover, combined treatment with DDR2 blockade and CD28xMUC1 BsAbs significantly increased the recruitment of SOC-specific T cells, granzyme B release, caspase-3 activation, and cytokine production.

CONCLUSIONI

DDR2 blockade mitigates collagen-mediated immune exclusion and enhances the efficacy of CD28xMUC1 BsAb-based immunotherapy in SOC models. These findings provide a mechanistic rationale for further investigation of combined DDR2 targeting and CD28×MUC1 costimulatory strategies to improve ovarian cancer immunotherapy.

B077

SELECTIVE ANTITUMOR EFFECTS OF A NOVEL RUTHENIUM COMPLEX IN NON-SMALL CELL LUNG CANCER MODELS

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INTRODUZIONE

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers and remains the leading cause of cancer-related mortality worldwide. Although platinum-based chemotherapy is widely used, its clinical efficacy is frequently limited by systemic toxicity and the development of drug resistance. Ruthenium-based metallodrugs have recently emerged as promising anticancer agents due to their selective activity and potentially improved safety profile. The aim of this study was to evaluate the antitumor effects of a novel ruthenium complex in NSCLC cellular models.

METODI

A newly synthesized ruthenium-based compound developed by the National Research Council (CNR) was investigated in A549 NSCLC cells and healthy lung fibroblasts (MRC5). Cell viability was assessed by MTT assay and compared with cisplatin-treated controls. Cell migration was evaluated using a scratch wound-healing assay, while cell invasive capacity was assessed by Transwell invasion assays. The biological activity of the ruthenium precursor lacking the active organic ligands was also examined. Molecular mechanisms underlying the observed effects were investigated by Western blot analysis of proteins involved in apoptosis and cell death pathways.

RISULTATI

Treatment with the ruthenium complex significantly reduced the viability of A549 cells, showing an antitumor effect comparable to cisplatin. In contrast, no significant cytotoxicity was observed in MRC5 fibroblasts at the same concentrations, indicating selective activity toward cancer cells. The ruthenium precursor lacking active ligands showed minimal biological activity, supporting the critical role of the final molecular structure. Furthermore, the compound significantly impaired tumor cell migration, as demonstrated by the scratch wound-healing assay, and reduced the invasive capacity of A549 cells in Transwell invasion assays. Western blot analysis revealed modulation of proteins associated with apoptotic pathways, suggesting activation of programmed cell death mechanisms.

CONCLUSIONI

The investigated ruthenium complex demonstrated selective antitumor activity against NSCLC cells while sparing healthy lung fibroblasts. In addition to reducing cell viability, the compound inhibited migratory and invasive behavior and induced molecular changes consistent with apoptosis. These findings support the potential of ruthenium-based compounds as promising alternatives to conventional platinum-based chemotherapy and warrant further preclinical investigation in NSCLC models.

B078

EXOSOMAL KDM2A AS A DYNAMIC LIQUID BIOPSY BIOMARKER IN HEPATOCELLULAR CARCINOMA

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INTRODUZIONE

Hepatocellular carcinoma (HCC) remains difficult to monitor, and validated circulating biomarkers predicting treatment response are lacking. KDM2A, a histone demethylase involved in oncogenic transcriptional control, may interact with the Notch3 pathway, which contributes to vascular and tumour biology. We aim to assess free and exosomal KDM2A and Notch3-related molecules in HCC liquid biopsies.

METODI

Plasma samples were collected at baseline, cycle 3 and cycle 5 from 28 HCC patients treated with atezolizumab/bevacizumab (Atezo/Beva) or STRIDE; 15 healthy subjects (HS) and 14 cirrhotic patients served as controls. Exosomes were isolated from 27 HCC patients and normalized to a pool of 9 HS. Exosomal KDM2A and Notch3 mRNAs were assessed by RT-qPCR, and proteins by western blotting. Free plasma KDM2A and Notch3 proteins were measured by ELISA in 28 HCC patients, 14 cirrhotic patients and 15 HS. Clinical variables included tumour burden, AFP, treatment type and radiological response. Statistical analyses included Mann-Whitney, Spearman, Friedman test with Bonferroni correction and ROC curves.

RISULTATI

Exosomal KDM2A mRNA was higher in HCC than in cirrhotic patients (FC=3.71; $p<0.0001$), with a stepwise increase from HS to cirrhosis and HCC ($p=0.03$). Levels decreased significantly from baseline/C3 to C5 ($p=0.007$), mainly in the Atezo/Beva group. KDM2A protein was undetectable in exosomes. Free KDM2A protein was higher in cirrhosis than in HS (median 470.9 vs 154.4 pg/mL) and discriminated viral from non-viral HCC etiologies (AUC=0.773; $p=0.03$), with no significant on-treatment changes. At baseline, circulating Notch3 correlated with AFP (Spearman $r=0.55$; $p=0.02$), whereas exosomal Notch3 mRNA/protein did not differ between HCC and HS and showed no association with clinical features.

CONCLUSIONI

Liquid biopsy profiling supports exosomal KDM2A mRNA as a promising biomarker in HCC, with potential value for treatment monitoring. Its reduction during therapy, particularly with Atezo/Beva, suggests a link with angiogenesis, including the Notch3 axis. Furthermore, exosomal mRNA KDM2A distinguishes aHCC etiologies, hinting at virus-driven pathways. Conversely, circulating and exosomal Notch3-related molecules appear less reliable as standalone clinical biomarkers.

B079

UNDERSTANDING THE METABOLIC PLASTICITY IN MALIGNANT PLEURAL MESOTHELIOMA FOR A NOVEL THERAPEUTIC APPROACH

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INTRODUZIONE

Malignant pleural mesothelioma (MPM) is an aggressive cancer usually diagnosed at an advanced stage and remains difficult to treat. It is classified as epithelioid (EM, ~50%), sarcomatoid (SM, ~15%), or biphasic (BM, ~25%), which combines EM and SM features. Median survival ranges from 12–24 months for EM to ~8 months for SM, with BM showing intermediate outcomes. Large-scale molecular studies highlight histological heterogeneity as a key factor underlying challenges in treatment response, and disease recurrence
Metabolic rewiring is a hallmark of cancer that supports cell proliferation, adaptation, and therapeutic resistance. MPM displays the Warburg phenotype, relying on glycolysis despite oxygen availability. However, the metabolic features of different MPM histotypes and their links to aggressiveness, treatment resistance, and diagnosis remain poorly understood. Here, we present a preliminary study investigating the effects of metabolic pathway inhibitors in EM and SM, aiming to identify novel therapeutic targets and potential diagnostic biomarkers.

METODI

We used murine AB1 (SM) and AB22 (EM) cell lines. The metabolic profile was determined using MRI. Energetic stress was induced using 2-Deoxy-D-glucose (2-DG) and Rotenone (Rot). We evaluated MTT viability, long-term growth curves, and clonogenicity assays. Western Blotting was used to quantify the expression of p-ERK 1/2, Hexokinase II (HKII), and GLUT-1.

RISULTATI

2-DG reduced proliferation in AB1 cells, while in AB22 cells exhibited a modest reduction. Rot induced a marked proliferative arrest in both cell lines. The dual treatment led to near-complete proliferative arrest, allowing cells to survive probably activating an alternative metabolic pathway. WB analysis revealed that 2-DG increased p-ERK 1/2 levels only in AB1 cells, indicating a compensatory survival mechanism. Rot upregulates HKII in AB1 cells, confirming a metabolic shift toward anaerobic glycolysis. In contrast, AB22 cells showed a decrease in HKII reflecting lower adaptive plasticity.

CONCLUSIONI

The AB1 SM cells display greater metabolic plasticity than AB22 EM cells. Developing therapeutic strategies based on energetic blockades may represent a promising approach to treat aggressive MPM, and preventing resistant phenotypes.

B080

TARGETING THE IL-33/ST2 AXIS: DESIGN AND BIOLOGICAL EVALUATION OF NOVEL SMALL-MOLECULE LIGANDS

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INTRODUZIONE

Interleukin 33 (IL-33) belongs to the family of "alarmins", which are danger signals secreted from damaged epithelial cells. IL-33, in particular, has a pivotal role in driving Th2-type adaptive immune response through the activation of innate lymphoid cells (ILC) in respiratory airways during allergic response and inflammation. In recent years, has emerged that the interaction of IL-33 and its receptor ST2 activate T regulatory cells in the tumor microenvironment (TME) leading to the suppression of CD8+ T cells activity. Thus, ST2 blockade could be exploited as immunotherapeutic target for promoting tumor regression. Accumulating evidence reveals that, in addition to the immunomodulatory role, IL-33/ST2 axis is implicated in regulating tumor growth, and metastasis in many cancers through the activation of MyD88 and TRAF-6, NF-κB, p38, JNK, and ERK pathways.
This study aims to design, synthesize, and biologically evaluate novel small-molecule ST2 ligands to modulate tumor immunosurveillance and cancer aggressiveness.

METODI

Using a structure-based drug discovery approach and in silico analysis, two potential ST2 ligands were rationalized and subsequently synthesized. Flow cytometry (FACS) was employed to screen ST2 expression in A549, DU145, PC3 and THP-1 cell lines in standard conditions or after TGF-β treatment (5 ng/ml). ERK1/2 phosphorylation was evaluated in cells exposed to IL-33 or to the newly synthesized ligands. Ligand cytotoxicity was assessed via MTT assays.

RISULTATI

Two candidate ST2 ligands were successfully synthesized. We found that: i) A549, DU145, and PC3 are ST2-positive cell lines; ii) ST2 expression is upregulated by TGF-β in a time-dependent manner in A549 and DU145; iii) IL-33-induced p-ERK 1/2 expression peaked at 15 and 45 min in DU145, and at 30 min in A549; iv) MTT assays showed low cytotoxicity across all tested concentrations; v) the synthesized ligands successfully modulated p-ERK 1/2 expression in DU145 cells.

CONCLUSIONI

These preliminary results show that ST2 expression in cancer cells may be modulated in the TME. Newly synthesized ligands display low cytotoxicity and regulate p-ERK 1/2 signaling. Future investigations will be conducted to evaluate ligand specificity using ST2-negative cell lines and an ST2 reporter gene assay.

B081

VALORIZATION OF AGRI-FOOD WASTE: ANTI-INFLAMMATORY PROPERTIES OF POMEGRANATE PEEL-DERIVED EXTRACTS

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INTRODUZIONE

Pomegranate peel, a major by-product of the juice industry, represents a sustainable source of bioactive phytochemicals with potential anti-inflammatory activity. This study evaluated the anti-inflammatory properties of three WB23S fractions obtained from pomegranate peel: decoction (D), supernatant (S) and pellet washing (W).

METODI

The activity of WB23S fractions was evaluated in RAW264.7 murine macrophages and CaCo-2 human intestinal epithelial cells stimulated with LPS or IL-1ß. Cells were treated with WB23S fractions, punicalagin, or ellagic acid for 24 hours. Cytotoxicity was assessed by MTT assay, nitric oxide production by Griess assay, iNOS and COX2 protein expression by Western Blot, and IL-1ß, IL-6, TGF-ß, and TNF-α expression by rt-PCR.

RISULTATI

All fractions showed good tolerability. Concentrations of 25, 50, 100 and 200 μg/ml were selected for biological evaluation. All fractions significantly reduced nitrite production in RAW264.7 cells, particularly at the highest concentration tested. Among the WB23S fractions, D fraction showed the strongest inhibitory effect on both iNOS and COX2 expression, whereas the W and S fractions reduced only iNOS expression to a lesser extent compared with the D fraction.
Phytochemical analysis revealed punicalagin (α+ß) and ellagic acid as the major compounds in WB23S fractions. The D fraction was enriched in punicalagin, whereas the W fraction showed higher ellagic acid levels. Ellagic acid showed no anti-inflammatory activity, while punicalagin displayed only moderate effects, suggesting that minor compounds may also contribute to the anti-inflammatory activity of the phytocomplex. Since chronic gastrointestinal inflammation may contribute to tissue damage and colorectal cancer development, the effect of WB23S fractions were investigated in intestinal epithelial cells. All fractions reduced cytokine expression in IL-1ß stimulated CaCo-2 cells.

CONCLUSIONI

These findings support the potential nutraceutical value of WB23S fractions, particularly the Decoction fraction, as natural anti-inflammatory agents. Further studies are needed to evaluate phytocomplex stability during gastrointestinal digestion and to better clarify the molecular mechanisms underlaying its biological activity.

B082

SINGLE-CELL TRANSCRIPTOMIC MAPPING REVEALS CELLULAR AND MICROENVIRONMENTAL HETEROGENEITY IN TRIPLE NEGATIVE BREAST CANCER BRAIN METASTASES

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INTRODUZIONE

Triple-negative breast cancer (TNBC), accounting for approximately 15% of breast cancers (BC), is the most aggressive subtype characterized by early metastasis and recurrence. It exhibits marked heterogeneity and a dynamic tumor microenvironment (TME), where cancer-associated fibroblasts (CAFs) play key regulatory roles in tumor progression. Despite frequent metastasis to visceral organs and the brain, access to metastatic brain tissue is limited due to the invasiveness of neurosurgical procedures. In this context, single-cell RNA sequencing (scRNA-seq) enables high-resolution profiling of tumor and TME heterogeneity in both primary and metastatic lesions.

METODI

Public scRNA-seq data from primary and brain-metastatic TNBC (Gene Expression Omnibus), and a brain metastatic sample processed in our laboratory, were integrated using Seurat v5 and Harmony, yielding approximately 109,000 cells post-quality control. Following cell clustering and annotation, mesenchymal cells underwent sub-clustering, functional enrichment, and cellcommunication analyses. Additionally, malignant epithelial cells were profiled for copy number variations (CNV) and gene regulatory network activity.

RISULTATI

scRNA-seq of primary and brain metastatic TNBC revealed marked cellular heterogeneity and transcriptional rewiring between the two sites. Primary tumor cells showed an enrichment of Activator Protein-1 (AP-1) signaling, whereas metastatic cells exhibited increased chromatin remodeling activity and higher malignant CNV scores. Immune cells, particularly T- and B-cells, were reduced in metastases, whereas mesenchymal populations were enriched. In this regard, mesenchymal cell sub-clustering identified diverse CAF phenotypes, including antigen-presenting, matrix, inflammatory, and proliferating CAFs. Importantly, antigen-presenting CAFs were enriched in primary tumors, whereas matrix CAFs were enriched in metastases. Moreover, functional analyses revealed distinct site-specific pathways across CAF subsets.

CONCLUSIONI

Overall, our high resolution scRNA-seq dissection provides new insights into the complex cellular and molecular landscape of brain metastases of TNBC. In particular, our data may suggest that stromal reprogramming is a key component of TNBC brain metastatic evolution providing novel potential targets for therapeutic intervention.

B083

TRANSCRIPTOMIC PROFILING AND FUNCTIONAL CHARACTERIZATION OF BREAST CANCER-ASSOCIATED FIBROBLASTS FOLLOWING MICROPLASTIC EXPOSURE

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INTRODUZIONE

Microplastics (MPs) are emerging environmental contaminants detected in both normal and tumor tissues. Increasing evidence suggests that MPs may contribute to breast cancer (BC) progression through oxidative stress, chronic inflammation, endocrine disruption, and altered cellular signaling. While most studies focused on the direct effects of MPs on BC cells, little is known about their impact on stromal components of the breast tumor microenvironment (TME). Among these, cancer-associated fibroblasts (CAFs) are key regulators of tumor progression involved in extracellular matrix (ECM) remodeling, inflammation, immune modulation, and metastasis. This study investigated the effects of short- and long-term MP exposure on CAF biology and transcriptional programs.

METODI

Normal fibroblasts (NFs), CAFs, and MDA-MB-231 cells were used as experimental models. CAFs were isolated from BC specimens and immortalized by CDKN2A knockdown and ectopic hTERT expression. Cell viability assays evaluated NFs and CAF responses to short-term (72 h) MP exposure. Transcriptomic changes were assessed by RNA sequencing and enrichment analyses. Functional effects of acute MP exposure were investigated through migration assays, whereas conditioned media (CM) from chronically exposed CAFs were used to evaluate effects on BC cell growth.

RISULTATI

MP exposure exerted distinct effects on NFs and CAFs. Short-term treatment reduced NF viability, whereas CAF growth was unaffected. Acute MP exposure upregulated ECM remodeling genes in CAFs, indicating early stromal activation, and increased CAF migration. In contrast, chronic MP exposure induced a sustained inflammatory transcriptional profile with enrichment of pathways related to arachidonic acid metabolism. Functionally, CM from chronically exposed CAFs significantly increased MDA-MB-231 proliferation, supporting the acquisition of a pro-tumorigenic secretory phenotype and indicating that long-term MP exposure promotes paracrine CAF-cancer cell communications.

CONCLUSIONI

MPs induce time-dependent transcriptional and functional reprogramming of breast CAFs. Short-term exposure promotes ECM remodeling and cell motility, whereas chronic exposure drives a sustained inflammatory phenoptype that may enhance tumor-supportive activity. These results identify CAFs as underappreciated targets of MP exposure and suggest that stromal alterations may contribute to MP biological effects in the BC TME.

B084

CEACAM5 BIOMARKER CHARACTERIZATION AND FLUORESCENT DETECTION TOOL FOR IMAGE GUIDED GASTRIC CANCER SURGERY

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INTRODUZIONE

Gastric cancer (GC) is the third leading cause of cancer-related mortality worldwide, with approximately one million new cases diagnosed annually. Although surgery remains the primary treatment, the lack of specific intraoperative tools to distinguish malignant from healthy tissue and to accurately identify metastatic lymph nodes necessitates indiscriminate lymph node resection, increasing surgical risk. Near-infrared (NIR, ~800 nm) fluorescent contrast agents such as indocyanine green (ICG) enable real-time visualization during laparoscopic and robotic procedures; however, their limited tumor specificity highlights the urgent need for biomarker-targeted fluorescent probes. Accordingly, this study aims to develop a novel fluorescence-based tool for real-time surgical use to improve metastatic mapping in gastrectomy procedures.

METODI

We evaluated the expression of candidate GC markers, including carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), in GC cell lines by western blotting. CEACAM5 expression was further assessed by immunohistochemistry (IHC) in tumor and matched healthy tissues from 35 GC patients who underwent partial gastrectomy at the Digestive System Surgery Unit, Florence.

RISULTATI

CEACAM5 was significantly expressed in tumor samples but absent or minimally present in healthy mucosa ($p = 0.0018$), confirming its tumor specificity. This specificity was further validated in patient-derived organoids. We are developing a novel tool targeting CEACAM5 and fluorescent in the NIR range to selectively label GC cell surfaces. The binding properties of this tool will be evaluated by western blotting in GC cell lines and in patient-derived tumor and healthy mucosal tissue slices.

CONCLUSIONI

Moving forward, this system represents a strong candidate for in vivo validation and clinical translation in precision gastrectomy, with potential applicability extending beyond gastric cancer to a broad range of CEACAM5-overexpressing malignancies.

B085

MODULATION OF CELL DEATH-RELATED GENES IN MALIGNANT MESOTHELIOMA CELLS TREATED WITH THE ONCOLYTIC ADENOVIRUS DL922-947

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INTRODUZIONE

Malignant pleural mesothelioma (MPM) is an aggressive lesion with limited therapeutic options. We have shown that the oncolytic adenovirus dl922-947 reduced cell viability, altered cell cycle progression, and induced immunogenic cell death in MPM cell lines and modulated angiogenic signaling through VEGF-A and IL-8 regulation. Furthermore, dl922-947 shows anti-tumor efficacy in murine xenograft models reducing tumor growth and enhancing survival. Moreover, its combination with cisplatin potentiated antitumor activity. In this study, we investigated the effects of dl922-947 in human MSTO mesothelioma cells, focusing on the modulation of cell death-related pathways.

METODI

MSTO cells were treated with dl922-947 for 24h at its IC50 (0,3 pfu/cell) evaluated with MTT assay. Global transcriptomic changes were analyzed by RNA sequencing (RNA-seq). Validation of selected genes was performed by RT-PCR. SIRT1 expression was assessed at different time points (6–24 h post-infection) by Real Time PCR.

RISULTATI

RNA-seq analysis revealed a significant modulation of pathways involved in programmed cell death and cellular stress responses. Enrichment analysis highlighted the activation of apoptotic and TNF-related signaling pathways, involving genes such as ABL2, LRP8, and SIRT1. Conversely, pathways associated with DNA damage response and repair, including ATM/ATR signaling, were downregulated, with reduced expression of key genes such as RAD51 and FANCD2.
RT-PCR validation demonstrated a progressive upregulation of SIRT1 from 6 to 24 hours after viral infection.

CONCLUSIONI

Treatment with dl922-947 induces a profound transcriptional reprogramming in MSTO mesothelioma cells, promoting pro-apoptotic signaling while suppressing DNA repair pathways. The upregulation of SIRT1 suggests a potential role as a key regulator of the cellular response to oncolytic virus infection and tumor cell survival mechanisms. These findings support the therapeutic potential of oncolytic adenoviruses in malignant mesothelioma and identify SIRT1 as a relevant molecular node for further investigation.

B086

EXTRACELLULAR VESICLES AND TRANSCRIPTOMIC SIGNATURES AS CANDIDATE BIOMARKERS OF ANTHRACYCLINE-INDUCED ENDOTHELIAL INJURY: PRELIMINARY FINDINGS FROM THE CARMA STUDY

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INTRODUZIONE

Anthracyclines are effective antineoplastic agents whose use is limited by cardiovascular toxicity. Endothelial dysfunction and premature senescence are increasingly recognized as early mechanisms of anthracycline-induced vascular damage. Extracellular vesicles (EVs) released by injured endothelial cells may contribute to vascular dysfunction and represent a source of circulating biomarkers. This study aimed to establish an in vitro model of doxorubicin-induced endothelial senescence and investigate cellular and vesicle-associated molecular alterations.

METODI

Human aortic endothelial cells (HAECs) were exposed to doxorubicin and maintained in recovery medium. Cellular senescence was assessed by senescence-associated β-galactosidase staining. Conditioned media were collected for EV isolation. RNA-seq was performed on endothelial cells, while untargeted proteomic analyses were carried out on both cellular and EV fractions. Bioinformatic integration was used to identify pathways associated with anthracycline-induced endothelial dysfunction.

RISULTATI

Doxorubicin induced a marked senescence-associated phenotype, with SA-β-gal positivity reaching up to 99%. Western blot analyses demonstrated acquisition of a prothrombotic endothelial phenotype, characterized by altered expression of regulators of endothelial hemostatic function. Doxorubicin-treated cells showed increased EV production compared with untreated controls. EVs were successfully isolated for proteomic characterization. Preliminary RNA-seq analyses revealed alterations in pathways related to cellular senescence, DNA damage response, inflammatory signaling, oxidative stress, mitochondrial homeostasis and endothelial function. Proteomic analyses of cellular and vesicular fractions were completed and are currently undergoing integrative evaluation to identify candidate molecular signatures associated with anthracycline-induced vascular injury.

CONCLUSIONI

Doxorubicin induces endothelial senescence, promotes a pro-inflammatory and prothrombotic phenotype, and increases EV release. These changes are associated with transcriptomic remodeling involving pathways linked to vascular aging, inflammation and cellular stress, supporting a senescence-associated endothelial phenotype. Integrated transcriptomic and proteomic analyses may provide novel insights into the mechanisms underlying anthracycline-induced cardiovascular toxicity and support the identification of biomarkers for early detection and risk stratification of treatment-related vascular damage.

B087

BASELINE SERUM CYTOKINE SIGNATURES PREDICT CLINICAL RESPONSE TO MESENCHYMAL STROMAL CELL THERAPY IN KNEE OSTEOARTHRITIS

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INTRODUZIONE

Mesenchymal stromal cell (MSC) therapy is a promising disease-modifying strategy for knee osteoarthritis (OA). Clinical outcomes remain heterogeneous, suggesting that the host inflammatory microenvironment may influence treatment efficacy. This study investigated whether baseline serum cytokine profiles could predict clinical response following intra-articular MSC therapy.

METODI

We conducted a prospective observational study on 42 patients with Kellgren-Lawrence grade II–III knee OA undergoing autologous bone marrow-derived MSC transplantation. Peripheral blood samples were collected at baseline (T0). Serum levels of IL-1β, IL-6, TNF-α, and IL-10 were quantified using multiplex ELISA. Clinical response was evaluated at 6 months using the WOMAC score. Patients achieving a ≥30% reduction in total WOMAC score were classified as Responders.

RISULTATI

At 6 months, 27 of 42 patients (64.3%) met responder criteria. Baseline serum IL-6 levels were significantly higher in Non-Responders compared with Responders (11.1±4.6 vs 6.8±2.4 pg/mL; p=0.018). A higher baseline IL-10/TNF-α ratio was associated with greater WOMAC improvement (r=0.42, p=0.009). ROC analysis identified baseline IL-6 as a predictor of treatment failure (AUC 0.76, 95% CI 0.62–0.89). In multivariable logistic regression adjusted for age, sex, BMI and Kellgren-Lawrence grade, baseline IL-6 remained independently associated with non-response (OR 1.18, 95% CI 1.03–1.36; p=0.021).

CONCLUSIONI

Baseline systemic inflammatory status appears to influence the clinical response to intra-articular MSC therapy in knee OA. Elevated serum IL-6 and lower IL-10/TNF-α ratios were associated with poorer outcomes. These findings support the potential role of serum cytokine profiling as a minimally invasive tool for patient stratification and precision regenerative medicine.

B088

INVESTIGATING RADIOIMMUNOTHERAPY COMBINATIONS TO MAXIMIZE THERAPY RESPONSE AND ANTI-TUMOR IMMUNE ACTIVATION

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INTRODUZIONE

Immunotherapy has transformed the treatment for cancer patients, yet resistance remains a major obstacle. Radiotherapy (RT) has emerged as a promising approach to modulate the tumor microenvironment, enhance antigen presentation, and boost adaptive antitumor immunity. By inducing the release of damage-associated molecular patterns (DAMPs) and tumor cell surface expression of MHC-I and PD-L1 molecules, RT can reactivate systemic anti-cancer immune response and improve immunotherapy efficacy. Understanding RT dosing and scheduling dynamics is crucial to optimize radioimmunotherapy synergy. This study aims to assess different RT protocols to maximize tumor immunogenicity, induce anti-cancer immune activation, and test the synergy with immunotherapy.

METODI

Tumor cell lines of various origins - including cutaneous melanoma, glioblastoma, osteosarcoma, breast, lung, gastric, pancreatic, and renal carcinomas - were treated with RT ranging from 2 to 6 Gy and assessed for PBMC recruitment via transwell migration assay. Immune checkpoint inhibitors (ICI) and RT were administered as single or combined therapies to 3D tumor cell-PBMC co-cultures. Calcein/PI staining was used to assess tumor viability, while qPCR was used to determine cytotoxicity-related immune signature following RT-ICI treatment. Immunophenotyping of PBMCs isolated from ICI-treated melanoma and NSCLC patients was performed by flow cytometry before and after RT to detect any anti-tumor immune reprogramming following RT.

RISULTATI

We observed that conditioned medium from RT-treated cancer cells recruits PBMCs in a dose-dependent manner. Calcein/PI staining of 3D tumor-immune cell co-cultures and qPCR analysis revealed that the RT-ICI combination exerts higher cytotoxicity and induces higher expression of anti-cancer immune activation markers than the single treatments. These in vitro data are strengthened by the augmented anti-tumor immune cytotoxic signature - associated with CD3+CD8+ and NK cells - detected in PBMCs isolated from ICI-treated patients following RT.

CONCLUSIONI

Overall, these data underscore how RT drives anti-tumor immunity and enhances tumor clearance, reinforcing the clinical potential of optimized radioimmunotherapy strategies for solid tumors.

B089

DECIPHERING METABOLIC DYSFUNCTION THROUGH SYSTEMS BIOLOGY. FROM MOLECULAR NETWORKS TO PRECISION MEDICINE

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INTRODUZIONE

Metabolic dysfunction is a hallmark of numerous chronic diseases, including obesity, insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease, and age-related disorders. Increasing evidence indicates that these conditions arise from complex interactions among mitochondrial dysfunction, immune activation, inflammatory signalling, and metabolic reprogramming. Understanding these interconnected mechanisms is essential for identifying biomarkers associated with disease susceptibility and progression, and therapeutic response.

METODI

Our research applies integrative systems biology approaches to investigate the molecular networks involved in metabolic dysfunction across diverse pathological conditions. Publicly available transcriptomic and proteomic datasets are analyzed using advanced bioinformatic methodologies, including differential expression analysis, functional enrichment, network reconstruction, and protein-protein interaction modelling. Particular attention is placed on pathways linking energy metabolism, mitochondrial function, immune regulation, oxidative stress and cellular adaptation.

RISULTATI

To further characterize disease-associated molecular signatures, large-scale interactome resources and multiplex assays are integrated to explore protein interaction networks and to identify key regulatory hubs involved in the regulation of metabolic homeostasis. These network-based analyses enable the characterization of complex molecular interactions that contribute to chronic disease development and progression.

CONCLUSIONI

Overall, these approaches provide deeper insights into the molecular mechanisms underlying metabolic dysfunction, enable the identification of clinically relevant biomarkers, and pave the way for more personalized strategies for disease prevention and treatment.

B090

MAGNESIUM AND EMPAGLIFLOZIN PROTECT AGAINST CISPLATIN-INDUCED CYTOTOXICITY IN HEK-293 CELLS: PRELIMINARY EVIDENCE

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INTRODUZIONE

Cisplatin remains a cornerstone chemotherapeutic agent for the treatment of several solid tumors; however, dose-limiting nephrotoxicity significantly restricts its clinical use. Magnesium supplementation provides partial renal protection, while sodium-glucose cotransporter-2 (SGLT2) inhibitors, including empagliflozin, have recently emerged as potential nephrotective agents due to their antioxidant, anti-inflammatory, and mitochondrial-stabilizing properties. This study aimed to investigate whether magnesium and empagliflozin exert additive or synergistic nephroprotective effects against cisplatin-induced cytotoxicity in HEK-293 human kidney cells.

METODI

Human HEK-293 were exposed to cisplatin (5, 10, and 25 μM) and to six different combinations, containing magnesium (1–2 mM) and empagliflozin (1–100 nM). After 24 hours, cisplatin-containing media were replaced with fresh medium containing combination treatment only, in order to simulate a post-injury recovery phase. Cell viability was assessed at 24, 48, and 72 hours by MTT assay.

RISULTATI

Cisplatin reduced cell viability in a time-dependent manner, with the most evident cytotoxic effects at 48 and 72 hours. The combination of Mg 2 mM and empagliflozin 100 nM showed the strongest protective effect, partially restoring viability after exposure to cisplatin 25 μM at 72 h, compared with cisplatin alone, suggesting attenuation of delayed cisplatin-induced cellular injury. Lower-dose combinations showed weaker protective effects, suggesting e dose-dependent interaction. Notably, maintenance of magnesium and empagliflozin after cisplatin withdrawal appeared to favor cellular metabolic recovery during the post-exposure phase.

CONCLUSIONI

Combined magnesium and empagliflozin treatment attenuated cisplatin-induced cytotoxicity in HEK-293 human kidney cells and promoted post-exposure recovery. These preliminary findings support further investigation of magnesium–SGLT2 inhibitor combinations as multimodal nephroprotective strategies during cisplatin-based chemotherapy.

B091

TRI-DAP NOD1 LIGAND-DRIVEN REPROGRAMMING POTENTIATES TRAINED IMMUNITY PHENOTYPE IN PSORIASIS PBMCS

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INTRODUZIONE

Trained immunity, defined as the long-term functional reprogramming of innate immune cells after exposure to pathogen-associated molecular patterns, may contribute to chronic inflammatory diseases, but its role in psoriasis remains poorly defined. Since most trained immunity protocols are based on isolated monocytes, we used whole peripheral blood mononuclear cell cultures to preserve immune-cell crosstalk and better reflect the complexity of circulating immune responses in vivo. This study investigated whether PBMCs from patients with moderate-to-severe psoriasis display a trained immunity-like phenotype after exposure to canonical or clinically relevant microbial-derived stimuli.

METODI

PBMCs were isolated from patients with psoriasis, (53), and matched surgical controls, (37). Cells were primed for 24 h with β-glucan or Tri-DAP, rested, and restimulated on day 6 for 24 h with LPS or poly(I:C), respectively. TNF-α and IL-6 were measured by ELISA as readouts of trained immunity, LDH release was evaluated to exclude cytotoxicity, and lactate plus residual glucose in supernatants were quantified as metabolic indicators of glycolytic remodeling.

RISULTATI

Tri-DAP/poly(I:C) stimulation induced a robust trained immunity-like response in PBMCs from psoriatic patients, with enhanced TNF-α and IL-6 production compared with controls. In contrast, the canonical β-glucan/LPS sequence, typically associated with trained immunity in isolated monocytes, induced a tolerogenic profile in whole PBMC cultures. The Tri-DAP/poly(I:C) response was accompanied by increased lactate production relative to residual glucose levels, supporting glycolytic metabolic remodeling during innate immune reprogramming.

CONCLUSIONI

These findings suggest that PBMCs from patients with psoriasis exhibit a trained immunity-like phenotype that is preferentially revealed by combined bacterial and viral PAMP stimulation through Tri-DAP and poly(I:C). By preserving multicellular immune interactions, the PBMC-based approach may uncover disease-associated innate immune reprogramming that is not fully captured by conventional monocyte-centered protocols. The Tri-DAP/poly(I:C) model may therefore provide a pathophysiologically relevant platform to investigate how infection-associated microbial and viral cues contribute to inflammatory amplification in psoriasis. This research was funded by the European Union, Next Generation EU—PNRR M6C2—Investimento 2.1 Valorizzazione e potenziamento della ricerca biomedica del SSN. PNRR-MAD-2022-12376878.

B092

SKIN CELL VOLATILOME PROFILING UNDER INFLAMMATORY CONDITIONS: BRIDGING IN VITRO MODELS AND CLINICAL BIOMARKERS

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INTRODUZIONE

Human skin is a dynamic organ with intense metabolic activity, continuously releasing volatile organic compounds (VOCs) from the stratum corneum, skin glands and resident microbiota. Alterations in VOC profiles have been associated with inflammatory skin conditions, including psoriasis and chronic dermatoses, highlighting their potential as non-invasive biomarkers of disease activity and therapeutic response. Here, a novel analytical methodology is presented for the quantification of volatile and semi-volatile compounds in a cellular model mimicking inflammatory skin conditions.

METODI

An in vitro fibroblast model (NHDF) was exposed to LPS, Poly I:C, and TNF-α to recapitulate key features of cutaneous inflammation. After 6 hours, 0.8 mL of cell supernatants were transferred into 20 mL headspace vials and stored at −80°C. Volatile metabolites were extracted by headspace SPME and analyzed using GC×GC-TOFMS. An internal standard fiber preloading strategy ensured analytical reliability across three biological replicates. The same platform was applied to serum samples (0.5 mL) from psoriatic patients and healthy controls, derivatized to monitor secondary metabolites, aiming to bridge in vitro inflammatory signatures with circulating VOC profiles.

RISULTATI

The combined volatile profile revealed more than 1,900 distinct chemical features; approximately 500 were retained after removal of control and blank signals. Multivariate analysis identified treatment-specific patterns, with separation between stimulated and non-stimulated groups, suggesting stimulus-associated differences in the fibroblast volatilome, though chemical identities remain to be confirmed

CONCLUSIONI

This study establishes a robust analytical framework for characterizing the skin cell volatilome under inflammatory conditions. Translational extension to patient serum will enable comparison between in vitro signatures and circulating biomarkers in psoriatic individuals. Integrating immunometabolic screening with disease-specific VOC profiles may support novel biomarker identification and targeted therapeutic strategies for refractory inflammatory skin disorders.
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B093

MODULATION OF WNT SIGNALING IN THE CROSSTALK BETWEEN DORMANT COLORECTAL CANCER CELLS AND HEPATIC STROMAL CELLS

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INTRODUZIONE

The liver is the primary site of metastasis in colorectal cancer (CRC). Metastatic spread is influenced by both a favorable tissue microenvironment and the intrinsic plasticity of CRC cells. The Wnt/β-catenin signaling pathway, essential for liver homeostasis, is often aberrantly activated in CRC and promotes the survival, adaptation, and proliferation of metastatic cells within the liver niche. This study aims to investigate the interaction between dormant CRC cells and hepatic stromal cells, focusing on the modulation of Wnt ligands.

METODI

SW620 and HCT116 CRC cell lines (MSS and MSI, respectively) and HepG2 liver cells were used. Cell viability, metabolic activity, cell cycle progression, and extracellular vesicle (EV) release were assessed by colorimetric assays and flow cytometry (FACS) after serum deprivation and treatment with the Porcupine inhibitor IWP2 to inhibit Wnt ligand secretion. HepG2 cells were exposed to conditioned media from quiescent CRC cells. Wnt ligand expression was analyzed by qRT-PCR, and APC and β-catenin by Western blotting. Selected Wnt ligands were validated in 15 paired primary CRC and synchronous colorectal liver metastasis (CRLM) samples.

RISULTATI

The MSS and MSI cellular models of CRC exhibited distinct adaptive responses to starvation and treatment with IWP2, with differential regulation of gene expression for Wnt3a, Wnt5a, Wnt7b, and APC, as well as in the production of EVs. Hepatic stromal cells were significantly influenced by factors secreted by quiescent CRC cells, showing altered expression of Wnt ligands and β-catenin. In particular, Wnt7b emerged as a modulator of intercellular communication. Consistent with this finding, Wnt7b expression was higher in tissues with CRC liver metastases (CRLM) compared to the corresponding primary CRC samples.

CONCLUSIONI

While Wnt/β-catenin signaling have been implicated in colorectal cancer progression and metastasis, their role in the crosstalk between dormant CRC cells and the hepatic microenvironment remains poorly defined. Our findings demonstrate that dormant CRC cells interact with hepatic stromal cells through remodeling of the Wnt/β-catenin pathway, highlighting Wnt7b as a potential context-dependent driver of the hepatic microenvironment. These preliminary data warrant validations in more physiologically relevant models and larger clinical cohorts, together with functional studies to further define the role of Wnt7b in CRC-liver crosstalk.

B094

DISSECTING THE EPIGENOMIC AND TRANSCRIPTOMIC LANDSCAPES OF PEDIATRIC ACUTE MEYLOID LEUKEMIA USING MULTI-MODAL SINGLE-CELL SEQUENCING

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INTRODUZIONE

Relapse in acute myeloid leukemia (AML) remains a major clinical challenge, particularly in patients harboring alterations in the RAS signalling axis, including both canonical RAS mutations and upstream regulators. While these alterations suggest a shared oncogenic origin, the mechanisms driving relapse remain unclear. We hypothesized that persistent cellular programs, supported by epigenetic regulation, contribute to relapse.

METODI

We performed single-cell RNA sequencing (scRNA-seq) and single-cell ATAC sequencing (scATAC-seq) on paired diagnosis (DX) and relapse (RE) samples from pediatric AML patients with RAS-pathway alterations. Cells were annotated using reference-based approaches. Non-negative matrix factorization (NMF) was applied to identify transcriptional programs from scRNA-seq data. Program activity was compared across conditions, cell types, and patients. Chromatin accessibility and transcription factor (TF) activity were assessed using scATAC-seq and chromVAR.

RISULTATI

We identified two dominant and conserved transcriptional programs across patients: (i) a metabolic program and (ii) a stem/progenitor-like program associated with cytoskeletal and motility signaling. Both programs were present at diagnosis and persisted at relapse, indicating stable underlying cellular states. While their relative abundance shifted across cell types and patients, and conditions, these core programs were consistently maintained. Integration with scATAC-seq is being used to investigate the epigenetic mechanisms underlying the persistence of these programs, with preliminary analyses suggesting involvement of transcription factor networks such as NFY and AP-1/BATF.

CONCLUSIONI

Relapse in RAS-driven AML is characterized by persistence and modulation of pre-existing cellular programs rather than the emergence of new states. Epigenetic maintenance of these programs may underlie treatment resistance, highlighting stable regulatory circuits as potential therapeutic targets.

B095

A NOVEL ROLE FOR NBS1 IN THE REGULATION OF NUCLEAR F-ACTIN DYNAMICS

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INTRODUZIONE

Actin dynamics, a key determinant of cell architecture, has recently emerged as an important regulator of nuclear functions, including the DNA damage response (DDR). Following genotoxic stress, nuclear actin reorganization promotes the spatial coordination of double-strand breaks (DSBs) and the recruitment of repair factors. A central regulator of this process is cofilin-1 (CFL-1), whose actin-depolymerizing activity is inhibited by LIM kinase (LIMK)-mediated phosphorylation. NBS1, a core component of the MRN complex, is essential for DSB sensing and repair. Hypomorphic NBS1 mutations cause Nijmegen Breakage Syndrome (NBS), a rare disorder characterized by genomic instability and severe immunodeficiency resulting from defective V(D)J recombination and class-switch recombination

METODI

The LIMK1/CFL-1 pathway was analysed in NBS1-depleted and control cells by Western blotting. UV-induced nuclear actin polymerization was monitored by live-cell imaging in U2OS cells stably expressing a GFP-tagged nuclear actin chromobody following RNAi-mediated NBS1 depletion.

RISULTATI

NBS1 depletion reduced LIMK1 expression, leading to decreased CFL-1 phosphorylation, enhanced CFL-1 activity, and aberrant F-actin depolymerization. Given the interplay between cytoplasmic and nuclear actin networks, we investigated whether NBS1 also regulates nuclear F-actin dynamics. NBS1 depletion decreased the proportion of F-actin-positive cells, delayed nuclear F-actin assembly, and shortened its persistence following UV damage. These effects correlated with reduced LIMK1 and phospho-CFL-1 levels, indicating increased CFL-1 activity. Similar alterations were observed in NBS lymphoblastoid cells, supporting the relevance of this pathway in a disease context.

CONCLUSIONI

Our findings identify NBS1 as a novel regulator of nuclear F-actin dynamics through the LIMK1/CFL-1 pathway. This function may contribute to the spatial organization and efficient repair of DSBs, and its disruption could play a role in NBS pathogenesis. Ongoing studies are investigating the contribution of nuclear F-actin remodelling to the resolution of programmed DSBs in lymphocytes and its potential as a therapeutic target in NBS.

B096

PREADIPOCYTES AS A NOVEL COMPONENT OF THE TUMOR MICROENVIRONMENT REINFORCING Y537S MUTANT BREAST CANCER MALIGNANCY

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INTRODUZIONE

Among estrogen receptor alpha gene mutations, Y537S, predominantly detected in metastatic breast cancer patients with shorter overall and progression-free survival, have the strongest functional impact and are key drivers of endocrine resistance. ESMO guidelines recognize them as biomarker and actionable therapeutic targets. Tumor progression is further shaped by bidirectional interactions between cancer cells-tumor microenvironment(TME), particularly preadipocytes, which critically influence therapeutic response and disease outcome. Moving from our previous finding demonstrating how Y537S mutation display intrinsic properties to interact with fibroblasts, in the present study we investigated how Y537S mutant cells may interfere adipocyte dedifferentiation.

METODI

Indirect co-culture experiments performed using MCF-7 parental(P) and Y537S(YS) CRISPR-expressing cells and murine 3T3L1 preadipocytes. Effects on proliferation(anchorage-dependent/independent assays),motility and invasiveness (Transwell migration and invasion assays) were assessed, and mRNA expression was analyzed by qPCR.

RISULTATI

Exposure of YS to 3T3L1 conditioned medium(CM) significantly increased mutant colony formation and invasion, effects not observed in P cells. On the other hand, the exposure of 3T3L1 to YS-CM induces a coordinated phenotypic switch: proteomic profiling reveals upregulation of mevalonate/cholesterol biosynthesis enzymes (LSS, NSDHL, FDPS), downregulation of cell-cycle regulators (CDK1, CDK2, NCAPD2) and CASP3, along with PPARγ gene induction and limited proliferation, likelihood related an early dedifferentiation state, acquiring enhanced migratory and invasive capacity sustained by enhanced gene expression of ACTA2, MMP2-9 and Resistin.

CONCLUSIONI

Collectively, the preadipocytes actively contribute to tumour progression of YS mutant cells through paracrine signaling. On the other hand, YS-CM, compared to P-CM, reprograms the preadipocytes towards a functionally activated, myofibroblast-like and pro-migratory phenotype. Our results evidence the important role of preadipocyte in TME shedding light on the underlying crosstalk mechanism between tumor-stroma. This perspective may provide novel tools able to inhibit TME-driven pathways sustaining tumor growth and invasiveness burden alongside the mutant ER.

B097

A FOUR-CIRCULATING MIRNA SIGNATURE REVEALS MOLECULAR MECHANISMS UNDERLYING FIBROSIS PROGRESSION AND LUNG ADENOCARCINOMA DEVELOPMENT IN IDIOPATHIC PULMONARY FIBROSIS PATIENTS

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INTRODUZIONE

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease with heterogeneous clinical trajectories and an increased risk of lung adenocarcinoma. Reliable minimally invasive biomarkers reflecting disease stage and molecular activity are still lacking. Circulating microRNAs (ci-miRNAs), due to their stability in biological fluids, represent promising candidates for monitoring fibrotic progression and malignant transformation.

METODI

Small RNA sequencing was performed on plasma samples from patients with early IPF (E-IPF), advanced IPF (A-IPF), lung adenocarcinoma (LC), IPF-associated lung adenocarcinoma (IPF-LC) and healthy controls (HCs). Differential expression analysis, functional enrichment, unsupervised clustering, and multivariate ROC modelling were used to identify disease-associated signatures and evaluate diagnostic performance.

RISULTATI

We identified 236 differentially expressed ci-miRNAs between E-IPF and A-IPF, indicating progressive molecular dysregulation during disease evolution. Enrichment analyses highlighted pathways involved in cytoskeletal organization, extracellular matrix/supramolecular fibre assembly, cell adhesion and growth factor signalling, consistent with tissue remodelling and fibrosis progression. Distinct ci-miRNA profiles were also observed in LC and IPF-LC, suggesting additional alterations linked to tumorigenesis within the fibrotic microenvironment. Unsupervised clustering showed overlap between E-IPF and HCs, whereas A-IPF, LC and IPF-LC formed distinct molecular clusters. Integrative analysis identified a four-miRNA signature (hsa-miR-10b-5p, hsa-miR-423-5p, hsa-miR-1246, hsa-miR-3184-3p) progressively upregulated across fibrotic stages and neoplastic evolution. These miRNAs are implicated in epithelial plasticity, extracellular matrix remodelling and tumor-microenvironment interactions, supporting their role in fibrotic-to-neoplastic transition. A multivariate model based on this signature accurately discriminated E-IPF from A-IPF (AUC = 0.983) and A-IPF from HCs (AUC = 0.844).

CONCLUSIONI

ci-miRNA profiles reflect a molecular continuum underlying IPF progression toward lung adenocarcinoma. The four-miRNA signature captures key pathogenic processes associated with fibrotic and neoplastic evolution. These findings support circulating miRNAs as promising minimally invasive biomarkers for disease staging and risk stratification in IPF and IPF-associated lung cancer, after validation in independent cohorts.

B098

ANALYSIS OF T CELL IMMUNE RESPONSE IN SUBJECTS WITH LATENT MYCOBACTERIUM TUBERCULOSIS INFECTION, SUFFERING FROM CHRONIC INFLAMMATORY DISEASES

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INTRODUZIONE

Patients with immune-mediated inflammatory diseases (IMIDs) undergoing biologic disease-modifying antirheumatic drug (bDMARD) therapy are at increased risk of reactivation of latent Mycobacterium tuberculosis (Mtb). This study aimed to evaluate the impact of bDMARD treatment on the functional and phenotypic profiles of T cell subsets involved in maintaining Mtb latency.

METODI

A longitudinal study was conducted on LTBI-positive patients with chronic inflammatory joint diseases, evaluated before treatment initiation (T0), and after 3 (T3) and 9 months (T9) of therapy. Peripheral blood mononuclear cells were stimulated with Mtb-specific antigens and phosphoantigens and analyzed by flow cytometry to assess cytokine production (IFN-γ) and cytotoxic mediators (perforin, granzyme B, granulysin) in $CD4^+$, $CD8^+$, and $\gamma\delta$ T cell subsets.

RISULTATI

$CD4^+$ T cells maintained a predominantly effector memory phenotype, with a transient reduction in IFN-γ production at T3, likely related to anti-TB prophylaxis, followed by recovery at T9. Cytotoxic function was preserved, with increased granulysin production over time. $CD8^+$ T cells showed stable frequencies and enhanced antigen-specific responses during treatment, maintaining or increasing IFN-γ and cytotoxic molecule production. $\gamma\delta$ T cells exhibited increased frequencies but reduced cytotoxic activity at T9, reaching levels comparable to LTBI controls.

CONCLUSIONI

Overall, bDMARD therapy does not impair the functional capacity of conventional T cells involved in Mtb control, while modulating $\gamma\delta$ T cell cytotoxicity. These findings suggest that targeted therapies, in the context of appropriate LTBI prophylaxis, may preserve immune mechanisms critical for maintaining Mtb latency.

B099

KDM3B INHIBITION REMODELS ERα-ASSOCIATED CHROMATIN AND TRANSCRIPTIONAL PROGRAMS IN ER-POSITIVE BREAST CANCER

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INTRODUZIONE

Endocrine therapy (ET) resistance remains a major unmet clinical need in estrogen receptor alpha-positive (ERα+) breast cancer (BC). Emerging evidence suggests that adaptive epigenetic reprogramming enables tumor cells to survive therapy-associated oxidative and transcriptional stress, thereby promoting disease progression. Among the epigenetic mechanisms potentially involved in these adaptive responses, histone lysine demethylases (KDMs) have emerged as candidate regulators of chromatin and transcriptional programs in BC, but the contribution of specific enzymes such as KDM3B, an H3K9me1/2 demethylase, remains poorly defined.

METODI

KDM3B was investigated in endocrine-sensitive and -resistant ER+ BC models and patient-derived organoids (PDOs) through genetic and pharmacological approaches, including siRNA-mediated silencing and treatment with the selective inhibitor PFI-90. Functional assays were integrated with transcriptomic and chromatin profiling approaches to characterize molecular and phenotypic changes associated with KDM3B targeting, including the evaluation of oxidative stress responses.

RISULTATI

Following PFI-90 treatment, KDM3B inhibition was accompanied by increased H3K9me2 accumulation and chromatin remodeling, including reduced accessibility at regulatory regions enriched for ERα-associated transcription factor motifs, supporting a role for KDM3B in maintaining ERα-driven transcriptional networks. Transcriptomic analyses showed downregulation of estrogen-responsive, cell cycle, and DNA repair pathways, together with modulation of oxidative stress- and ferroptosis-associated programs. Functionally, KDM3B blockade impaired cell growth and reduced long-term proliferative capacity, including in PDOs. In parallel, KDM3B inhibition increased intracellular reactive oxygen species (ROS) levels and promoted accumulation of DNA damage. Notably, KDM3B targeting also altered the response of resistant models to endocrine therapy.

CONCLUSIONI

Collectively, our findings indicate that KDM3B contributes to the regulation of ERα-associated chromatin and transcriptional programs linked to oxidative stress responses and endocrine therapy response in ER+ BC cells, supporting its further investigation as a potential epigenetic target in endocrine resistance.

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B100

IDENTIFICATION OF A NOVEL SCN5A COMPLEX ALLELE IN A FAMILY WITH BRUGADA SYNDROME

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INTRODUZIONE

Brugada syndrome (BrS) is an inherited arrhythmogenic disorder characterized by marked clinical and genetic heterogeneity. Pathogenic variants in SCN5A gene account for approximately 30% of cases, while the genetic basis remains unknown in most of affected families. We report the molecular characterization of a BrS family carrying a novel SCN5A variant, providing a putative complex allele.

METODI

A 64-year-old male proband was referred for genetic testing after a positive flecainide test showing a type 1 Brugada ECG pattern. Family history revealed two sons with type 1 Brugada pattern (one with syncope), one daughter with negative flecainide testing but episodes of second-degree atrioventricular block, sister and niece positive for type 1 Brugada pattern after flecainide test, and a brother and the father with sudden cardiac death. Targeted next-generation sequencing (NGS) was performed using a commercially available capture-based panel (ExtCas, Sophia Genetics) including 128 genes associated with inherited cardiac channelopathies and cardiomyopathies.

RISULTATI

Genetic analysis identified in our proband a novel heterozygous SCN5A frameshift variant, c.1663dup p.(Glu555Glyfs*166), absent from the literature and ClinVar database, and classified as likely pathogenic according to ACMG criteria. Additional heterozygous variants of uncertain significance (VUS) were detected in SCN5A [c.3911C>T p.(Thr1304Met)], SCN10A [c.2T>C p.(Met1?)], PKP2 [c.464G>C p.(Ser155Thr)] and DSP [c.1103T>C p.(Ile368Thr)]. Segregation analysis demonstrated the presence of the likely pathogenic SCN5A variant in the proband's sister and daughter. The niece carried both the familial likely pathogenetic variant and the SCN5A c.3911C>T p.(Thr1304Met) variant. Subsequent testing confirmed the co-occurrence of both SCN5A variants in the proband's sister and daughter.

CONCLUSIONI

Segregation analysis showed that SCN5A c.1663dup p.(Glu555Glyfs*166) and SCN5A c.3911C>T p.(Thr1304Met) consistently co-segregate in all investigated carriers. These findings suggest the presence of a previously undescribed complex SCN5A allele that may contribute to the BrS phenotype in this family. Further studies are required to determine the allelic phase and functional impact of these variants.

B101

FROM HYPERTENSION TO COGNITIVE IMPAIRMENT: SORTILIN AS A NOVEL BIOMARKER

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INTRODUZIONE

Arterial hypertension is a major risk factor for dementia, driving cerebrovascular dysfunction and oxidative stress. Additionally, hypertension contributes to the development of carotid artery stenosis (CAS), further exacerbating cognitive decline. The precise role of sortilin, a protein recently implicated in cardiovascular and neurodegenerative disease, remains to be clarified. Therefore, we aimed to assess the potential role of sortilin in the molecular mechanism linking hypertension and cognitive impairment

METODI

C57BL/6J mice were implanted with osmotic minipumps releasing recombinant sortilin protein or vehicle and monitored for blood pressure (BP) levels.In vitro, human brain microvascular endothelial cells (HBMECs) were treated with Angiotensin II (Ang II) and pre-transfected with SORT1 siRNA; endothelial function was measured by Matrigel tube formation.We recruited 53 hypertensive patients with CAS, stratified by disease severity, who underwent endarterectomy at the University Hospital of Salerno. Explanted plaques were analyzed for SORT1 and OSTEOCALCIN mRNA, collagen (Masson's trichrome), lipid content (Oil Red O), and sortilin colocalization with α-SMA and von Willebrand factor by immunofluorescence. Plasma levels of sortilin and malondialdehyde (MDA) were analyzed, and cognitive status was evaluated using the Mini-Mental State Examination (MMSE).

RISULTATI

Chronic sortilin infusion raised arterial BP and increased brain 3-NT, 4-HNE, GFAP, and ICAM-1, while reducing VE-cadherin. In HBMECs, SORT1 silencing prevented Ang II–induced VE-cadherin loss, while plasma from CAS patients inhibited endothelial tube formation in a sortilin-dependent manner.In plaques, higher SORT1 and OSTEOCALCIN expression correlated with advanced CAS; histology showed increased collagen and lipid deposition, with sortilin colocalizing with α-SMA and von Willebrand factor. Notably, circulating sortilin and MDA were elevated in severe CAS and inversely correlated with MMSE scores.

CONCLUSIONI

Our data highlight a potential role for sortilin as a novel biomarker and therapeutic target in hypertension-associated vascular damage and possibly in cognitive impairment.

B102

NUCLEAR TAU MODULATES DNA DAMAGE RESPONSE IN AGGRESSIVE PROSTATE CANCER VIA ß-CATENIN/PARP1

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INTRODUZIONE

Prostate cancer (PCa) is a highly heterogeneous malignancy that expresses Tau protein (MAPT). Although traditionally associated with its cytosolic accumulation in tauopathies, Tau exhibits a subcellular distribution that includes the nucleus, where it has been suggested to play a protective role. ß-catenin, a key player in tumor progression, acts in the nucleus by regulating genes involved in cell proliferation and the DNA damage response. These processes are coordinated by PARP1, which promotes DNA repair or, under persistent stress, triggers apoptosis. This study aims to clarify the nuclear role of Tau in PCa by evaluating its involvement in DNA integrity and its potential interaction with the ß-catenin/PARP1 signaling axis.

METODI

Prostate cancer cell lines representing different stages of tumor progression (LNCaP, DU145, PC3) were analyzed for Tau expression by immunoblotting and RT-qPCR. Subcellular localization of Tau and ß-catenin was assessed by immunofluorescence and differential protein extraction. Their interaction was investigated in DU145 cells through MAPT silencing and co-immunoprecipitation. The response to cisplatin-induced genotoxic stress was evaluated by measuring DNA damage (p-H2AX), apoptosis (PARP1 cleavage), and the expression of Tau, p-Tau, ß-catenin, and p-ß-catenin.

RISULTATI

Tau expression was higher in aggressive PCa phenotypes, both in total and phosphorylated forms and across multiple isoforms and was associated with increased ß-catenin levels. In these cell lines, both proteins showed enhanced nuclear localization. Transient Tau silencing in DU145 cells reduced ß-catenin levels and induced PARP1 cleavage, indicating apoptosis activation. Co-immunoprecipitation confirmed the association between Tau and ß-catenin in the nuclear compartment. Cisplatin treatment led, in a time-dependent manner, to reduced Tau and ß-catenin levels, alongside increased p-H2AX, and PARP1 cleavage, consistent with DNA damage and apoptosis.

CONCLUSIONI

In aggressive prostate cancer, increased expression and nuclear co-localization of Tau and ß-catenin suggest functional cooperation supporting tumor progression. The loss of nuclear Tau homeostasis disrupts this balance, leading to DNA damage accumulation, ß-catenin inactivation, impaired PARP1-mediated DNA repair, and increased apoptosis. Overall, Tau emerges as a key regulator of DNA integrity and cell fate, representing a potential therapeutic target.

B103

HEPATOCYTE DEPLETION OF ERK5 IMPAIRS THE RESPONSE TO LIPOTOXIC OXIDATIVE STRESS RESULTING IN DEFECTIVE INSULIN RECEPTOR SIGNALING

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INTRODUZIONE

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a leading cause of chronic liver disease, with insulin resistance representing a pivotal event. However, mechanisms regulating hepatocyte insulin signaling during lipid overload remain incompletely understood. Extracellular signal-regulated kinase 5 (ERK5), highly expressed in hepatocytes and implicated in liver cancer, may also play a role in metabolic regulation. Here, we investigated the contribution of ERK5 to hepatocyte insulin sensitivity.

METODI

Mice with hepatocyte-specific deletion of ERK5 (ERK5ΔHep) and control mice were fed with a high-fat diet (HFD) for 16 weeks. Glucose tolerance test (GTT) was performed by injection of glucose i.p. (1 g/kg), and insulin tolerance test (ITT) with insulin (0.8 U/kg). A murine hepatocyte cell line (MMH) was silenced using lentiviral vectors encoding shRNA for the ERK5 gene. Mitochondrial depolarization was assayed using the TMRE staining protocol. Quantification of total ROS using DCFDA and of mtROS using MitoSOX. OXPHOS metabolism was measured by Seahorse.

RISULTATI

Murine hepatocytes (MMH) were silenced for ERK5 using shRNA and exposed to palmitic acid before insulin stimulation to resemble lipotoxicity in vitro ERK5 depletion reduced insulin receptor (IR) expression and abolished Akt activation. Additionally, ERK5 silencing induced JNK activation, leading to inhibitory phosphorylation of IRS-1 (Ser307). In ERK5-deficient cells, increased mitochondrial reactive oxygen species (ROS) were observed, accompanied by reduced expression of NRF2 target genes HMOX1 and NQO1, indicating impaired antioxidant defence. Treatment with the antioxidant N-acetylcysteine restored IR levels and prevented IRS-1 inhibitory phosphorylation. ERK5 loss also resulted in mitochondrial dysfunction, shown by reduced membrane potential and impaired oxidative phosphorylation, together with upregulation of PGC-1α and TRIB3. In vivo, hepatocyte-specific ERK5 knockout mice (ERK5ΔHep) fed a high-fat diet displayed impaired glucose tolerance and reduced insulin sensitivity, associated with decreased hepatic IR expression and increased PGC-1α and TRIB3 levels.

CONCLUSIONI

Overall, ERK5 preserves hepatocyte insulin sensitivity by sustaining an NRF2-dependent antioxidant response and limiting JNK-mediated IRS-1 inhibition, maintaining Akt signaling under metabolic stress.

B104

MULTIMODAL IN VIVO IMAGING IDENTIFIES SPECIFIC PATHOPHYSIOLOGICAL CHARACTERISTICS OF THE TUMOR MICROENVIRONMENT IN A MOUSE MODEL OF PANCREATIC CANCER.

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INTRODUZIONE

The tumor microenvironment (TME) is crucial in pancreatic ductal adenocarcinoma (PDAC) progression.A key feature is the desmoplastic stroma, which promotes tumor growth, tissue stiffening, and reduced drug delivery.High-frequency ultrasound (HFUS) and photoacoustic imaging (PAI) enable non-invasive evaluation of tumor structure and function in vivo.Using HFUS/PAI, we assessed the remodeling processes characterizing the PDAC microenvironment.

METODI

Orthotopic PDAC xenografts were generated in nude mice via ultrasound-guided injection of 1×10# cells, forming four groups (PANC-1, RLT-PSC, and 1:1 and 1:5 co-injections). Longitudinal imaging with Vevo F2-LAZR-X assessed growth (HFUS), perfusion (CEUS), oxygenation (PAI), and stiffness (SWE), followed by histology, RNA-seq, and RT-qPCR of ECM genes.

RISULTATI

In an initial set of experiments, orthotopic PANC-1 tumors were compared with healthy pancreas. Increased tumor stiffness was observed (p=0.003), which positively correlated with collagen deposition and negatively with oxygen saturation (sO_2%) and microvascular density (MVD). We then analyzed tumors generated from the four injection conditions described in the Methods. Heterotypic tumors, particularly those derived from PANC-1/RLT-PSC (1:5), exhibited slower growth, lower oxygenation, and reduced perfusion, consistent with a more hypoxic microenvironment. Histological analyses showed homogeneous collagen distribution in heterotypic tumors, whereas homotypic tumors displayed predominantly peripheral collagen deposition. RT-qPCR demonstrated increased expression of collagen I, III, IV, and fibronectin in heterotypic tumors, indicating stellate cell-driven extracellular matrix remodeling. RNA-seq revealed distinct transcriptional profiles between homotypic and heterotypic tumors, with enrichment of extracellular matrix organization, collagen formation, and fibril assembly pathways, particularly in the PANC-1/RLT-PSC (1:5) group. Interferon signaling was also enriched in RLT-PSC-containing tumors, suggesting a pro-inflammatory stromal contribution. Finally, liver metastases were observed in both 1:1 and 1:5 heterotypic groups, indicating enhanced metastatic potential associated with stellate cells.

CONCLUSIONI

Overall, stellate cells enhanced ECM remodeling and promoted a hypoxic, mechanically stiff tumor microenvironment, which favours the metastatic process in PDAC.

B105

TARGETING THE INFLAMMATORY MICROENVIRONMENT IN PDAC: HOW TUMOR-STROMA CROSSTALK AND VEGF INHIBITION FUEL COMPENSATORY SURVIVAL PATHWAYS AND CHEMORESISTANCE

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INTRODUZIONE

Pancreatic Ductal Adenocarcinoma (PDAC) is characterized by an extensive desmoplastic reaction and an immunosuppressive microenvironment, contributing to its unfavorable prognosis. Cancer-associated fibroblasts (CAFs) and proinflammatory cytokines play a pivotal role in modulating tumor progression and shielding malignant cells from chemotherapy. This study aimed to characterize the inflammatory profile of PDAC and evaluate how the crosstalk between epithelial cells and CAFs influences survival and chemoresistance.

METODI

Gene expression analysis was performed on PDAC and normal-appearing pancreatic tissue samples. Primary and co-culture models were established to study the tumoral spatial organization. Cell viability and apoptosis were assessed in PDAC cell lines treated with Gemcitabine and anti-VEGF Bevacizumab, either as monotherapy or in combination. Immunohistochemistry for α-Smooth Muscle Actin (α-SMA) was used to evaluate the activation of the periductal stroma.

RISULTATI

Molecular analysis revealed a significant overexpression of VEGF, IL-1ß, IL-6 and TGF-ß in PDAC samples compared to normal tissues. Ex vivo cultures showed a distinct growth pattern, with clusters of epithelial cells closely surrounded by CAFs. IHC confirmed the high expression of α-SMA in the periductal region of PDAC samples, indicating a robust activation of pancreatic stellate cells into myofibroblastic CAFs. Apoptosis assays showed that Bevacizumab autonomously increased cell viability in PDAC cells under standard culture conditions, even when combined with Gemcitabine.

CONCLUSIONI

The high levels of TGF-ß and the periductal α-SMA expression confirm a highly activated desmoplastic stroma, acting as a physical and biochemical barrier. The upregulation of proinflammatory cytokines (IL-1ß, IL-6) likely promotes an autocrine and paracrine prosurvival loop. Our findings reflect a known paradox in PDAC: even in vitro under standard conditions, VEGF inhibition directly triggers cell-autonomous compensatory survival pathways, mirroring the hypoxia-induced chemoresistance observed in vivo. Targeting this inflammatory profile and the CAF-mediated protective niche remains a critical challenge for developing more effective translational strategies to overcome Gemcitabine resistance in PDAC.

B106

M-CSF ROLE IN ENDOTHELIAL–MONOCYTE CROSSTALK IN CELLULAR SENESCENCE AND INFLAMM-AGEING

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INTRODUZIONE

Senescent endothelial cells (senECs) contribute to tissue dysfunction by secreting high levels of pro-inflammatory mediators. Among these, colony-stimulating factors (CSFs) can play a role recruiting monocytes in inflammatory niches. Indeed, macrophage colony-stimulating factor (M-CSF) is elevated in chronic inflammatory conditions, from ageing to neurodegenerative diseases. M-CSF regulates monocyte survival, proliferation, differentiation and migration. However, its role in modulating monocyte adhesion to senECs remains under-explored. This study aims to investigate M-CSF involvement in endothelial–monocyte/macrophages communication and interaction, and its potential role in promoting monocytes adhesion to endothelial wall in inflammageing.

METODI

Primary human umbilical vein endothelial cells (HUVECs), THP-1 monocytic cells and THP-1-derived macrophages (M0) were used. THP-1 cells and M0 were exposed for 48h to either recombinant M-CSF or endothelial cell-conditioned media (CM). Endothelial cells were treated for 48h with CM derived from THP-1 cells or M0 previously stimulated with M-CSF. Expression of M-CSF, its receptor CSF-1R, and inflammatory and adhesion molecules was evaluated at mRNA and protein levels by RT-qPCR, immunoblotting and flow cytometry.

RISULTATI

Senescent ECs, but not young ECs, showed increased M-CSF protein expression. Exposure to senEC-derived CM induced CSF-1R upregulation in THP-1 and M0 ($M\phi$s). Moreover, stimulation with either recombinant M-CSF or senEC-derived CM increased the expression of inflammatory and adhesion molecules in $M\phi$s, including TNF-α, IL-1β, CXCL10 and CD62L. Conversely, senECs exposed to CM from M-CSF-treated $M\phi$s displayed enhanced expression of MCP-1, ICAM-1 and VCAM-1, whereas no significant changes were observed in young ECs.

CONCLUSIONI

Senescent endothelial cells represent a relevant source of M-CSF, which promotes a pro-inflammatory and pro-adhesive phenotype in monocytes/macrophages. In turn, activated macrophages further enhance endothelial inflammatory activation, suggesting the existence of a feed-forward loop that may contribute to monocyte adhesion and vascular inflammation during inflammageing.

B107

FROM AN INCONCLUSIVE TARGETED GENETIC TESTING TO A MOLECULAR DIAGNOSIS: THE ADDED VALUE OF WHOLE EXOME SEQUENCING IN NEONATAL EPILEPTIC ENCEPHALOPATHY.

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INTRODUZIONE

Developmental and epileptic encephalopathies (DEEs) are a group of neurological disorders characterized by early-onset seizures, developmental impairment, and drug resistance. The application of “genome-wide” next-generation sequencing (WES) has significantly improved the diagnostic yield in neonatal epilepsy, especially in unresolved cases with targeted gene panels.

METODI

A 2-month-old infant admitted to the Neonatal Intensive Care Unit for refractory epileptic encephalopathy underwent genetic testing using a targeted NGS panel including 10 genes associated with neonatal epileptic disorders. Due to the absence of a conclusive molecular diagnosis, the analysis was subsequently extended to WES.

RISULTATI

Targeted panel analysis identified a heterozygous variant in KCNQ2, classified as a Variant of Uncertain Significance (VUS), insufficient to explain the severe clinical phenotype. WES revealed three pathogenic heterozygous variants: SZT2 c.8062C>T p.(Arg2688*), CR2 c.593dup p.(Leu198Phefs14), and C8B c.1282C>T p.(Arg428). In addition, a homozygous SCN1A intronic variant, c.603-91G>A, classified as drug response, was identified. Moreover, the truncating SZT2 variant is of particular interest given the established role of SZT2 in mTOR pathway regulation and its association with developmental and epileptic encephalopathies, intellectual disability, and treatment-resistant seizures. In addition, the pathogenic variants identified in CR2 and C8B genes, involved in immune regulation and complement activation, suggest a potential contribution of neuroinflammatory mechanisms as disease modifiers. Furthermore, the homozygous SCN1A pharmacogenomic variant may have contributed to the marked resistance to antiepileptic treatment observed in this patient.

CONCLUSIONI

The presented case highlights the added diagnostic value of WES in newborns with suspected DEE and inconclusive targeted NGS results. The findings support a complex oligogenic model in which variants affecting neuronal signaling, immune pathways, and drug response may collectively contribute to disease severity and pharmacoresistance. Comprehensive genomic approaches can improve diagnostic accuracy and provide clinically relevant insights into the biological mechanisms underlying severe neonatal epileptic encephalopathies.

B108

ANTI-FIBROBLAST ANTIBODIES FROM IGG4-RD PATIENTS MODULATE FIBROBLAST FUNCTION IN VITRO

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INTRODUZIONE

IgG4-related disease (IgG4-RD) is an uncommon fibroinflammatory disease characterized by extensive tissue fibrosis, lymphoplasmacytic infiltration, obliterative phlebitis, and enrichment of IgG4-secreting plasma cells in affected tissues. Increasing evidence suggests that B cells actively contribute to fibrogenesis, as fibroblast activation and collagen accumulation correlate with tissue infiltration by IgG4-positive plasma cells and regress following B-cell depletion therapy. These findings support the existence of a pathogenic crosstalk between B cells and stromal cells in sustaining tissue remodeling.
This project seeks to define the role of antibodies generated by IgG4-RD-derived B cells in promoting fibroblast activation and fibrotic tissue responses.

METODI

Total IgG were obtained from IgG4-RD patients and healthy controls by means of Protein G affinity chromatography. Recombinant monoclonal antibodies (rmAb) were generated from IgG4-RD patients B cells by single cell PCR and expression vector cloning. Primary human dermal fibroblasts (HDF) were isolated from normal skin of subjects undergoing abdominoplasty. The binding capacity of IgGs and rmAb to HDF was evaluated by immunofluorescence and western blotting followed by mass spectrometry and immune-enzymatic assay. Their role in modulating cytokine production and migration of fibroblast was evaluated by ELISA and scratch wound assay respectively. A proteome profile array was also used to assess the activated pathway on fibroblast.

RISULTATI

The rmAb produced have different V gene usage with the clonotype IGKV1-39 as the predominant one. Immunofluorescence staining with patient-derived monoclonal antibodies show that 4/8 display immunoreactivity to normal skin fibroblast. Western blotting analysis on HDF extracts showed reactivity with proteins in the range of 35-38 KDa that were analysed by mass spectrometry and identified as Annexin 2A, reactivity further confirmed by patient's purified total IgG.
Stimulation of dermal fibroblasts with either patient IgGs of rmAb induced cytokines production and promoted fibroblast cell migration compared to control IgGs. Notably, fibroblast effector function were promoted via the activation of ERK 1/2 pathway.

CONCLUSIONI

These results suggest that IgG4-RD patients may develop anti fibroblast antibody directed against Annexin A2 that are able to modify fibroblast behaviour, potentially driving the fibro-inflammatory process in IgG4-RD patients

B109

A ZINC FINGER-NFATC3-MYC AXIS LINKS GUT MICROBIOTA WITH INTESTINAL TUMORIGENESIS

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INTRODUZIONE

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide, with increasing incidence and a high clinical burden. Emerging evidence highlights a relevant role for gut microbiota dysbiosis in CRC initiation and progression; however, the molecular mechanisms linking microbial-derived signals to tumor-promoting pathways remain poorly understood. We previously identified the zinc finger protein cellular nucleic acid-binding protein (CNBP) as a tumor-promoting factor in brain tumors. CNBP also regulates the oncogene MYC, a major driver of tumorigenesis in several cancers, including CRC. Here, we investigated CNBP as a mediator of microbiota-driven colorectal tumor progression and evaluated its potential as a therapeutic target.

METODI

The clinical relevance of this axis was assessed using patient-derived datasets and tumor samples. In vitro and in vivo models, including CRC cell systems, AOM–DSS and APC^loxP mice, and intestinal organoids, were used to investigate the response of this signaling cascade to microbiota-derived stimuli through molecular and gene expression analyses.

RISULTATI

We demonstrate that the CNBP/NFATc3 complex promotes CRC tumorigenesis by acting as a sensor of microbiota-derived signals and driving MYC transcription. Mechanistically, CNBP binds MYC G-quadruplex structures in cooperation with NFATc3, thereby enhancing MYC expression. Gut microbiota, particularly adherent-invasive Escherichia coli (AIEC), induce CNBP activation through dephosphorylation at serine 49 (S49), promoting its interaction with NFATc3, nuclear translocation, and subsequent MYC transactivation. Consistently, CNBP S49 dephosphorylation was detected in human CRC tissues and was induced by lipopolysaccharide (LPS) and microbiota-derived signals. Functionally, intestinal epithelial cell-specific CNBP knockout impaired tumorigenesis in both inflammation-associated and genetically driven CRC models, reducing tumor burden, polyp formation, and metastatic dissemination. Furthermore, administration of an orally bioactive antisense oligonucleotide (ASO) targeting CNBP suppressed tumor growth in mice and in human and mouse intestinal organoids, while improving overall survival in APC^loxP mice.

CONCLUSIONI

Our study identifies a novel microbiota-driven CNBP/NFATc3/MYC axis that promotes CRC progression through MYC transcriptional activation. CNBP S49 dephosphorylation represents a key regulatory switch and highlights this pathway as a potential therapeutic target in CRC.

B110

PROGNOSTIC AND PREDICTIVE VALUE OF NUCLEAR HO-1 IN HER2+/HR+ BREAST CANCER PATIENTS TREATED WITH NACT

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INTRODUZIONE

Heme Oxygenase-1 (HO-1) plays a key role in cell adaptation to stressors through its antioxidant, antiapoptotic, and anti-inflammatory properties. Its overexpression favors tumor aggressiveness and therapy resistance. In this work, we study HO-1 as a prognostic/predictive biomarker in patients with HER2+/HR+ breast cancer.

METODI

The study was approved by the Regional Ethics Committee (CER-Liguria, cod. ONC-HO1) and adheres to the Declaration of Helsinki. Samples and clinical data were retrospectively collected from 38 patients with HER2+/HR + breast cancer who received neoadjuvant chemotherapy (NACT) before surgery. HO-1 expression was assessed via immunohistochemistry (IHC) on pre-NACT biopsies and scored from 0 (negative) to 3+ (strong). Correlations between HO-1 and clinical parameters (pathologic complete response [pCR] and residual disease) were calculated using Spearman's correlation ($p < 0.05$). Receiver operating characteristic (ROC) analysis assessed the model's discriminatory ability.

RISULTATI

IHC showed that HO-1 was localized within nuclei, cytoplasm, and stroma. After NACT, 16 patients (42.1%) achieved a pCR. Nuclear HO-1 negatively correlated with therapeutic response ($r = -0.6221$, $p = 0.0001$) and positively with residual disease ($r = 0.5997$, $p = 0.0001$). No significant correlation was found for cytoplasmic or stromal HO-1. Univariate analysis showed that nuclear HO-1 expression predicts residual disease (OR = 10.2535, 95% CI: 2.1483–48.9378, $p = 0.04$). ROC curves confirming the ability to discriminate patients with or without residual disease showed the best performance for nuclear HO-1 (AUC = 0.8821, 95% CI: 0.7793–0.9849) compared to cytoplasmic or stromal expression. Joint evaluation of all three compartments did not significantly improve accuracy (AUC = 0.8920, 95% CI: 0.7916–0.9925).

CONCLUSIONI

These data indicate that nuclear HO-1 is closely linked to NACT resistance and disease progression in HER2+/HR+ breast cancer, representing a promising biomarker to early identify non-responding patients.

B111

CLINICAL IMPACT OF DPYD AND UGT1A1 GENOTYPING IN A COHORT OF CANCER PATIENTS FROM SICILY

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INTRODUZIONE

Fluoropyrimidines (FU) and SN-38–based therapies, including 5-fluorouracil, capecitabine, and irinotecan, are widely used in the treatment of solid tumours. However, 10-30% of patients (pts) experience severe treatment-related toxicity, potentially leading to treatment modification, hospitalisation, or death in up to 1% of cases. Germline variants in DPYD and UGT1A1, encoding key enzymes in drug metabolism, are well-established determinants of toxicity risk and treatment variability. Despite recommendations, their clinical implementation and impact require further evaluation in more comprehensive clinical settings

METODI

We conducted an analysis of oncological pts from Eastern Sicily undergoing pharmacogenetic testing between 2019 and 2024. The study included pts tested for DPYD (N=948) and UGT1A1 (N=1025). Clinical data comprised treatment exposure, adverse events (CTCAE v 5.0), and on-treatment modifications. Genotyping was performed using a validated assay (EasyPGX® Diatech), and analyses were conducted using STATA®v19.5. Logistic regression identified predictors of grade ≥2/≥3 toxicity and treatment adaptation, while Cox models assessed TTF and OS

RISULTATI

All pts initiated therapy with genotype-guided dose reduction according to AIOM-SIF 2024 guidelines. In the DPYD cohort, 11.9% carried one variant allele, mainly c.2194G>A (DPYD*6) with reduced activity in 2.9%. Among FU-treated pts, exposure was comparable across genotypes and DPYD status was not independently associated with grade ≥2 or ≥3 toxicity. Additional dose reductions were more frequent in variant carriers, particularly for grade ≥2 toxicity, while no consistent pattern was observed for grade ≥3 events. Treatment interruptions and discontinuations were mainly guided by toxicity severity.

In the UGT1A1 pts, genotype distribution was 44% wild-type, 44% heterozygous, and 12% homozygous. Among pts receiving SN-38–based therapies, toxicity did not differ across genotypes; however, neutropenia, including grade ≥3 events, showed a significant allele-dose relationship (OR 1.96). Treatment modifications were not associated with genotype and appeared to be driven by neutropenia. Genotype-guided dose individualisation did not impact treatment modifications, TTF, or OS.

CONCLUSIONI

Pharmacogenetic profiling of DPYD and UGT1A1 enhances treatment safety without compromising efficacy, while mitigating toxicity risk. These findings support the integrating of genotype-guided strategies into routine oncology care.

B112

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL VANILLIN-BASED TRIAZOLE DERIVATIVES WITH ANTICANCER ACTIVITIES

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INTRODUZIONE

Oxidative stress and the dysregulation of reactive oxygen species (ROS) play a significant role in cancer development and progression. Elevated ROS levels contribute to the maintenance of an aggressive tumor microenvironment, promoting cancer cell proliferation, disease progression and metastatic diffusion. Thus, natural bioactive products play critical roles in anticancer drug development. To enhance the antioxidant activity of natural compounds, we incorporated two antioxidant pharmacophores into a single molecular scaffold to improve free-radical scavenging capacity and provide multi-target biological activity. We used vanillin, a phenolic aldehyde naturally extracted from vanilla beans, and 1,2,4-triazole heterocyclic derivatives, which have emerged as a privileged pharmacophore with anticancer potential due to unique physicochemical properties, bioavailability, and free radical scavenge activity.
The synthesized compounds were evaluated for their anticancer activity against E0771 murine model of Triple Negative Breast Cancer cells and A549 human Non-small Cell Lung Cancer cells alone or in association with everolimus.

METODI

The synthesis of vanillin-based triazole derivatives was accomplished through a multi-step sequence: O-alkylation of vanillin with propargyl bromide in the presence of potassium carbonate and benzyl triethylammonium chloride. The reaction was conducted in anhydrous acetone, affording the corresponding propargyl ether, in good yield. This intermediate served as a key alkyne precursor for subsequent cycloaddition reactions, to reach the final compounds. Cell cytotoxicity was assessed via MTT assays.

RISULTATI

The three tested compounds (ZS8, ZS13, and ZS16) significantly inhibited A549 cell proliferation; however, no synergistic effect was observed when the compounds were combined with everolimus. In contrast, E0771 cells, which were more sensitive to everolimus only ZS8 significantly inhibited cell proliferation and exhibited a strong synergistic effect when used in combination with everolimus.

CONCLUSIONI

The combination treatment showed significant synergistic antiproliferative activity in E0771 cells, highlighting its potential as a breast cancer therapeutic strategy. Further investigations are needed to clarify the mechanisms of cell death responsible for the observed synergy.

B113

SYNBIOTIC COMBINATION OF BACILLUS MEGATERIUM DSM 32963 AND N-3 PUFA SALT COUNTERACTS INFLAMMATION IN COLONIC TISSUE AND DRIVES SYSTEMIC IMMUNOMODULATION IN VIVO IN ULCERATIVE COLITIS PATIENTS

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INTRODUZIONE

Ulcerative colitis (UC) is driven by chronic intestinal and systemic inflammation. Attempts to restore mucosal immunotolerance using omega-3 (n-3 PUFAs) often fail because patients cannot efficiently convert them into specialized pro-resolving mediators (SPMs). To bypass this, we developed SynΩ3, a synbiotic combining Bacillus megaterium DSM 32963 with n-3 PUFA lysine salt. This pilot study evaluates the immunomodulatory efficacy of SynΩ3 through a dual translational approach: ex vivo in human UC biopsies and in vivo in a placebo-controlled clinical trial.

METODI

Ex vivo, SynΩ3 were applied to colon biopsies from 14 UC patients and 6 healthy controls. Tissue cytokine expression was assessed by rt-PCR, and macrophage polarization (M1/M2) via immunofluorescence. In vivo, UC patients received either placebo or SynΩ3 (Verum) from baseline (T0) to T4. Placebo patients switched to Verum between T2 and T4. Systemic cytokines and peripheral immune cell phenotypes (monocytes/macrophages, T-cell subsets, and plasma cells) were monitored.

RISULTATI

Ex vivo, SynΩ3 was more effective than n-3 PUFA alone in treating ulcerative colitis (UC) biopsies, promoting the polarization of mucosal macrophages from an inflammatory M1 profile to an anti-inflammatory M2 profile, confirmed by decreased pro-inflammatory cytokines (IL-1β, IL-6, IL-23 and IFN-γ) and increased anti-inflammatory cytokines (IL-4 and IL-13). In vivo, baseline UC patients exhibited systemic inflammation (elevated IL-6, CCL3, activated CD4+CD25+ T cells and plasma cells). The placebo arm remained immunologically static. Conversely, Verum drove profound immunomodulation: by T4, it strongly suppressed adaptive immunity, reducing CD4+ TEM cells, activated T subsets, plasma cells, and cytokines (IL-1β, CCL3, IL-6, IL-12) while increasing anti-inflammatory IL-4. Placebo patients achieved similar immunological resolution after switching to Verum.

CONCLUSIONI

SynΩ3 bypasses defective patients metabolism to restore immune homeostasis in UC. By targeting mucosal macrophage polarization and strongly suppressing systemic T-cell activation, SynΩ3 represents a highly effective, clinically relevant adjuvant to conventional therapies.

B114

POLYSTIRENE MICROPLASTIC PARTICLES ENGAGE IGF1R SIGNALING IN BREAST CANCER

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INTRODUZIONE

Microplastics (MPs) are environmental pollutants generated from the fragmentation of larger plastic particles. Recent studies have assessed their presence in several human tissues, raising concerns on their potential pathogenetic role in human diseases (1-3). In this context, MPs are being investigated for their ability to act as cellular stressors and interfere with endocrine homeostasis in diverse hormone-dependent tumors (4-5). Herein, we set out to test whether MPs interfere with the insulin/insulin-like growth factor system (IIGFs) in breast cancer (BC), based on the well-recognized oncogenic role played by this signaling axis (6-7).

METODI

Estrogen Receptor-positive MCF-7 BC cells were stimulated with 1 μm fluorescent polystyrene beads (referred to as F-MPs), whose identity was characterized by FTIR spectroscopy. Immunofluorescence studies were performed to assess cellular uptake. Activation of IGF1R- dependent signaling and downstream effectors were evaluated in cells stimulated with F-MPs by immunoblotting. Gene silencing approaches, CRISPR/Cas9-mediated knockout, and pharmacological inhibition were used to confirm receptor dependency. Intracellular reactive oxygen species (ROS) production was assessed using fluorescent probes, and antioxidant rescue experiments were performed with N-acetylcysteine (NAC).

RISULTATI

Analyses of FTIR-derived spectra confirmed the chemical identity of F-MPs. MCF-7 cells cultured as both monolayer and 3D spheroids internalized MPs in a concentration-dependent manner. Exposure of MCF-7 cells to F-MPs triggered the activation of the IGF1R/IRS1 signaling cascade and its downstream transduction partners pERK/pAKT. These responses were halted by the pharmacological inhibitor OSI-906, as well as by silencing or knocking out IGF1R. Likewise, restoring IGF1R levels was sufficient to rescue pathway activation after F-MPs treatment. Of note, the engagement of the IGF1R pathway by F-MPs relied on ROS as demonstrated using the scavenger NAC.

CONCLUSIONI

MPs activate the IIGFs in BC cells through the involvement of ROS. Our findings provide preliminary insights into the potential oncogenic role played by MPs to be further investigated in ongoing studies.

References

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B115

DUAL TARGETING OF AR/FLNA AND CXCR4 SIGNALING RESTRAINS TUMOR GROWTH AND MICROENVIRONMENT REMODELING IN TRIPLE-NEGATIVE BREAST CANCER

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INTRODUZIONE

Triple-negative breast cancer (TNBC) accounts for 15–20% of all breast cancers and is characterized by the lack of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression. Despite significant advances in breast cancer treatment, chemotherapy remains the main systemic option for TNBC because of the limited availability of actionable targets. The androgen receptor (AR), expressed in 20–50% of TNBCs, has recently emerged as a potential driver of tumor progression. Here, we investigated the role of AR, its interactor Filamin A (FLNA), and the CXCR4 axis in TNBC progression and in the remodeling of the tumor microenvironment.

METODI

A combination of in vitro and in vivo approaches was used to evaluate the contribution of AR, FLNA, and CXCR4 to TNBC progression. AR signaling and its functional interactions were investigated in MDA-MB-231 and MDA-MB-453 cells by co-immunoprecipitation, Western blotting, immunofluorescence, BrdU incorporation, wound-healing, and transwell assays. The effects of tumor cells on the microenvironment were assessed using NIH3T3 fibroblasts. The effects of the Rh2025u peptide, which disrupts the AR/FLNA interaction, and of the CXCR4 inhibitor peptide R54 were evaluated both in vitro and in MDA-MB-231 BALB/c xenograft models.

RISULTATI

Androgen stimulation promoted TNBC cell proliferation. AR directly interacted with FLNA, and disruption of this complex by Rh2025u markedly reduced tumor growth in vivo and in vitro models. In parallel, inhibition of CXCR4 with R54 mainly affected the tumor microenvironment by reducing fibroblast activation, proliferation, and migration. These findings suggest that AR sustains TNBC progression through complementary mechanisms, driving tumor cell proliferation via FLNA while promoting tumor microenvironment remodeling through the CXCR4 axis.

CONCLUSIONI

AR promotes TNBC progression through complementary mechanisms involving FLNA and CXCR4. Targeting the AR/FLNA complex primarily impairs tumor cell growth, whereas CXCR4 inhibition mainly remodels the tumor microenvironment. These results identify AR-associated signaling networks as promising therapeutic targets and support the further preclinical development of Rh2025u and R54 for AR-expressing TNBC.

B116

DEVELOPMENT OF A TARGETED DRUG–NANOCARRIER FOR THE TREATMENT OF PANCREATIC CANCER: VALIDATION THROUGH A NOVEL 3D MICROFLUIDIC "TUMOUR-ON-CHIP" PLATFORM.

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INTRODUZIONE

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy characterized by poor prognosis and limited therapeutic options. Within the TRANSCAN-funded PaCaNano project, we developed gemcitabine phosphate nanoparticles (GemP-NPs) functionalized with the bispecific antibody scDb-hERG1-β1, targeting the hERG1/β1 integrin complex expressed in PDAC cells. Microfluidic tumor-on-chip platforms were used to reproduce key features of the PDAC microenvironment and evaluate the therapeutic potential of this targeted nanomedicine approach.

METODI

The scDb-hERG1-β1 antibody was produced and characterized by UNIFI and conjugated to GemP-NPs developed by KIT. Antibody integrity and binding activity before and after conjugation were assessed by peptide and cell-based ELISA assays. Tumor-on-chip platforms were used to culture pancreatic cancer cells under dynamic 3D conditions mimicking the native tumor microenvironment. Therapeutic efficacy was evaluated by live/dead imaging (Calcein-AM/Propidium Iodide), nanoparticle fluorescence tracking, and LDH release assays. The antitumor activity of scDb-hERG1-β1 was further assessed in orthotopic murine PDAC models by monitoring tumor growth and tumor volume.

RISULTATI

The scDb-hERG1-β1 antibody retained its binding activity after GemP-NP conjugation. Cell ELISA assays showed enhanced binding of functionalized nanoparticles to PANC1 cells, with negligible binding in control GD25 cells, confirming target specificity. Confocal microscopy demonstrated efficient cellular uptake of both unconjugated and conjugated nanoparticles. In the tumor-on-chip platform, scDb-hERG1-β1 significantly reduced tumor cell viability, as shown by decreased Calcein-AM fluorescence, increased Propidium Iodide staining, and elevated LDH release. In vivo, scDb-hERG1-β1 reduced tumor burden and volume in orthotopic PDAC models. Ongoing studies are assessing the biodistribution and therapeutic efficacy of scDb-hERG1-β1-functionalized GemP-NPs in desmoplastic orthotopic PDAC models generated by co-inoculation of PANC1 and pancreatic stellate cells.

CONCLUSIONI

These findings demonstrate the potential of hERG1/β1-targeted nanomedicine as a selective therapeutic strategy for PDAC. Furthermore, the combination of tumor-on-chip technology and imaging-based analyses provides a robust and scalable human-relevant platform for the preclinical evaluation of targeted therapies, supporting the translational development of innovative treatments for pancreatic cancer.

B117

GLP-1R DECLINE IN BREAST CANCER PROGRESSION IS REGULATED BY DNA METHYLATION

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INTRODUZIONE

Breast cancer (BC) is the most common oncological disease affecting women worldwide. Despite improvement in the survival rate over the past two decades, its incidence continues to rise every year. Several factors are associated with BC risk, among which metabolic syndrome plays a key role both in the onset and progression, affecting its incidence, prognosis and treatment response efficacy. Recently, the repositioning of antidiabetic drugs for cancer treatment has been investigated with encouraging outcome on cancer prevention, especially upon glucagon-like peptide-1 receptor agonists (GLP1R-As). Despite the clinical relevance of GLP-1R targeting, its involvement in BC is still poorly investigated. Here, GLP-1R expression regulation in BC is addressed.

METODI

GLP-1R contribution to BC tumorigenesis was analyzed in: 1) human BC cell line models, representing the main four BC subtypes; 2) specimens derived from two distinct primary BC patient cohorts at diagnosis; 3) an orthotopic BC in vivo model transplanted with GLP-1R overexpressing 4T1 cells.

RISULTATI

The Cancer Genome Atlas (TCGA) data sets and IHC analysis of a cohort of human BC samples showed an inverse correlation between GLP-1R expression and BC patient life expectancy, highlighting its prognostic value. Interestingly, immunoblotting analysis showed that GLP-1R protein levels are progressively down-modulated in more aggressive BC subtypes associated with worse prognosis. Notably, methylated DNA immunoprecipitation (MeDIP) analysis on a specific CpG site within GLP-1R promoter and treatments with either demethylating or methylating agents confirmed GLP-1R sensitivity to DNA methylation. Moreover, GLP-1R restoration was able to counteract proliferation, migration and invasiveness both in vitro and in vivo. These findings show that GLP-1R loss, as results of DNA methylation alteration, is involved in the acquisition of aggressiveness and metastatic potential through BC progression.

CONCLUSIONI

These findings reveal that GLP-1R down-modulation could represent one of the molecular players supporting higher aggressiveness in BC, as consequence of a DNA methylation-dependent regulation. GLP-1R might represent both a prognostic biomarker for longitudinal monitoring and a potential therapeutic target in invasive BC. Therefore, therapeutic approaches preventing its silencing could have interesting clinical implication in BC patient management.

B118

AMPHIPHILIC AND GANGLIOSIDE GM1-BINDING PROPERTIES OF SARS-COV-2 SPIKE GLYCOPROTEIN VARIANTS DISSECTED BY CHARGE-SHIFT ELECTROPHORESIS

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INTRODUZIONE

SARS-Cov-2 entry into target cells is mediated by the Spike glycoprotein (SARS-2-S). Both the binding of SARS-2-S to the ACE2 receptor and the fusion of the viral envelope with the membrane of target cells are crucial. Our aim was to compare the amphiphilic and GM1-binding properties of the main SARS-2-S variants, to dissect the role of amphiphilic interactions in SARS-2-S receptor-binding and fusogenic activities.

METODI

Charge-shift electrophoresis (CSE) of monomeric extra-cellular domain (ECD) of 2019-nCoV, delta (B.1.617.2), omicron (B.1.1.529), and omicron 4-5 (BA.4/BA.5) variants of SARS-2-S in 1% agarose at pH 9.0, with/without Triton X-100 (TX), alone or with anionic deoxycholic acid (DOC) or cationic cetyltrimethylammonium bromide (CTAB), or with ganglioside GM1. For the sizing of mixed GM1/SARS-2-S micelles we used CSE at various GM1:Spike molar ratios and Ferguson analysis of transverse polyacrylamide gradient gels. In silico amphiphilic profiling was performed by the Eisenberg's hydrophobic moment plot, based on SARS-2-S cryo-EM pdb structures (6vyb, 7sbl, 7tgw, 8gou) visualized in JMol.

RISULTATI

All SARS-2-S variants exhibited anodic shifts in TX/DOC and cathodic shifts in TX/CTAB, reflecting the formation of mixed detergent/SARS-2-S micelles. GM1/SARS-2-S micelles were also formed with all variants, whose anodic shifts correlated with the GM1:SARS-2-S molar ratios of 209:1, 208:1, 249:1, and >280:1 for 2019-nCoV, delta, omicron and omicron 4-5 SARS-2-S, respectively. Amphiphilic regions in the N-terminal domain (NTD) and at both extremities of the Receptor Binding Domain (RBD) of the S1 subunit consisted solely of beta-strands/sheets. Amphiphilic regions in the S2 subunit were mostly alpha-helices, coinciding with regions involved in the prefusion-to-post-fusion transition, including heptad repeats 1 (HR1), most of the S1/S2-S2' fragment with the 6-helix bundle 1 (6HB-1), and the 6HB-2 formed by the HR1 and the HR2.

CONCLUSIONI

All SARS-2-S variants exhibited distinct amphiphilic and varying GM1-binding properties. Amphiphilic beta-strands/sheets in the NTD and RBD of the omicron 4-5 variant were more evenly distributed than in the other variants. They might promote the detachment of the RBD from the NTD of the neighboring subunit of trimeric SARS-2-S and its transition to the "up" conformation required for the binding to the ACE2 receptor. Amphiphilic alpha-helices in the HR1 coiled coil and the 6HB-1 and 6HB-2 of the S2 subunit likely contribute to the fusogenic activity of SARS-2-S.

B119

EVOLUTION OF PARASITOLOGICAL DIAGNOSTICS: AN OPTIMIZED PROTOCOL FOR CLASSICAL COPROSCOPY

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INTRODUZIONE

Clinical pathology faces a challenge in microscopic parasitology due to low sensitivity from pre-analytical errors. Although molecular techniques are expanding, direct morphological identification remains the definitive proof of an active invasion. This study evaluates an optimized pre-analytical and analytical protocol designed to eliminate sample degradation and restore the reliability of classical coproscopy.

METODI

Refined over 15 years, the algorithm enhances the pre-analytical phase through three core interventions. First, multisampling and isolation: micro-samples are collected from 7–8 distinct sites to minimize missing unevenly distributed pathogens, isolating feces from toilet water to avoid osmotic shock in trophozoites. Second, oxygen and thermal protection: tight packing cuts off atmospheric oxygen lethal to anaerobic protozoa, while immediate aluminum foil wrapping forces a slow temperature decrease to prevent helminth disintegration. Third, enhanced analytical density: classical coproscopy (concentration/flotation) is systematically supplemented by permanent staining (modified Ziehl-Neelsen, trichrome) and targeted antigen tests, under double independent slide cross-verification. Efficacy was validated by cross-referencing microscopy against parallel PCR in a traditional cohort in 2007 (N=1024) and the optimized cohort in 2022 (N=1107).

RISULTATI

In the 2007 baseline study, traditional microscopy detected 107 positive cases compared to 358 by PCR (29.89% sensitivity). In the 2022 validation study using the optimized protocol, microscopy identified 484 positive cases, while parallel PCR detected 538 cases. The implementation of this pre-analytical rigor significantly reduced false-negatives, increasing microscopic diagnostic sensitivity to 89.96% relative to molecular detection.

CONCLUSIONI

Shielding fragile biological structures from osmotic, oxygen, and thermal shock prevents pre-analytical degradation. This protocol achieves 90% diagnostic accuracy from a single sample, successfully bridging the cost-effectiveness of classical coproscopy with molecular sensitivity standards.

B120

CHARACTERIZATION OF 3D PRIMARY THYROID CELL CULTURES DERIVED FROM HUMAN NORMAL AND TUMOR TISSUES

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INTRODUZIONE

Patient-derived three-dimensional (3D) culture models provide relevant systems to study normal tissue features and tumor biology by better preserving cell phenotype than conventional two-dimensional (2D) cultures. In thyroid research, maintaining a differentiated phenotype in vitro remains challenging, while single-cell RNA-seq studies have highlighted tumor heterogeneity, including distinct differentiation states. Here, we aimed to establish and characterize patient-derived 3D primary cultures from normal and tumor thyroid tissues to assess their ability to recapitulate thyroid differentiation and tumor-associated phenotypes.

METODI

Human thyroid tissue samples were obtained from patients undergoing thyroidectomy for thyroid nodules suspicious for malignancy after written informed consent and Ethics Committee approval (161/CEL Catania 2). Samples were enzymatically dissociated to obtain normal and tumor-derived primary thyroid cells, which were cultured under 2D and scaffold-free 3D conditions. Normal 3D cultures were compared with corresponding 2D cultures to assess thyroid differentiation/function markers, whereas tumor-derived 3D cultures were compared with patient-matched normal 3D thyrocytes to evaluate tumor-associated phenotypes. Marker expression was assessed by RT-qPCR and Western blot, and trends were compared with publicly available datasets.

RISULTATI

Compared with 2D cultures, normal 3D thyrocytes showed higher expression of thyroid differentiation/function-related markers, including TG, TPO, TSHR, SLC5A5, DUOX1, DUOX2, and IYD, supporting better preservation of a differentiated phenotype. Tumor-derived 3D cultures displayed reduced expression of thyroid differentiation markers compared with patient-matched normal 3D thyrocytes, with the lowest levels observed in the less differentiated tumor-like condition. Conversely, they showed increased expression of selected tumor-associated markers, including LGALS3, FN1, NOX4, and TIMP1. These trends were consistent with THCA data.

CONCLUSIONI

These preliminary results support the establishment of patient-derived 3D primary thyroid cultures as biologically relevant models to study normal and tumor cell phenotypes, with potential applications in investigating tumor initiation and progression, the effects of environmental contaminants, biomarker discovery, and therapeutic testing.

B121

LOST IN TRANSPORT: GENETIC VARIANTS OF THE CARRIER SLC22A4/OCTN1 SHAPE INTRATUMOR MICROBIOTA IN COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) results from a multifactorial pathogenic cascade where nutritional factors and a permissive intestinal microbiota intersect a predisposing genetic background. Understanding the molecular interactions among these components is imperative to facilitate disease prevention and optimize treatment from a precision medicine perspective.

METODI

We used qPCR to analyze the abundance of the CRC-associated pathobiont Fusobacterium nucleatum (Fn) and the commensal Escherichia coli (ECi) in a cohort of 99 primary colorectal tumors and paired adjacent normal mucosa. Patients were genotyped for the 503F genetic variant of the solute transporter SLC22A4/OCTN1, a carrier for diet- and microbe-derived organic compounds previously linked to the genetic risk of inflammatory bowel disease and early-onset sporadic CRC. To model the effect of the variant in vitro, “wild type” colorectal Cancer Stem Cells were transduced with OCTN1 503F and infected with Fn.

RISULTATI

F. nucleatum was similarly present in malignant and normal colon tissues; however, we observed that the F. nucleatum/E. coli ratio was significantly higher in tumorous tissues, indicating that an altered microbial ecology, more than the representation of a single bacterial species, is distinctive of the CRC microenvironment. Most importantly, the tumor-specific increase in the Fn-to-EC ratio varied across genotypes of the solute transporter SLC22A4/OCTN1: the difference in the Fn/EC ratio between tumor and normal tissues steadily increased in patients with the number of alleles of the disease-associated variant L503F (23.48, 159.56, and 211.03 for 0, 1, or 2 c1672t alleles; p=0.0215), suggesting a genetic influence over tumor-associated dysbiosis.
Mechanistically, compared to the parental line, mutant CSCs displayed a dampened innate immune response to Fn in vitro, alongside impaired autophagy and reduced clearance of intracellular bacteria. Moreover, consistent with its involvement in CSC reaction to pathogens, SLC22A4/OCTN1 was co-immunoprecipitated with the bacterial sensor and autophagy inducer NOD2 in 293T cells.

CONCLUSIONI

Thus, genetic variants of the intestinal carrier SLC22A4 alter bacteria-host communication and reshape the intratumor microbiota in favor of a pro-carcinogenic pathobiont by dampening innate immunity and increasing the tolerance of cancerous cells to bacterial invasion. The transported molecules responsible for these effects are currently being investigated.

B122

THE ROLE OF MITOCHONDRIAL CALCIUM SIGNALLING IN SKELETAL MUSCLE PATHOPHYSIOLOGY

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INTRODUZIONE

In skeletal muscle, mitochondrial calcium (mitCa2+) plays multiple roles, including regulating TCA cycle enzyme activity to sustain ATP production and controlling cell death pathways. The Mitochondrial Calcium Uniporter (MCU) is the highly selective channel responsible for mitochondrial calcium uptake. The importance of fine-tuning mitCa2+ uptake is demonstrated by the multi-organ disease phenotype of patients carrying loss-of-function mutations in a critical MCU regulatory subunit, namely MICU1.

METODI

In the mouse model, we modulated MCU activity both genetically, by developing skeletal-muscle specific MCU knockout animals, and pharmacologically, by the administration of small molecules targeting the MCU.

RISULTATI

We demonstrated that mitCa2+ uptake controls skeletal muscle mass, activity, and metabolism, both in adulthood and during ageing. Moreover, we have identified the natural polyphenol Oleuropein as a specific MCU activator that stimulates mitochondrial respiration via MICU1-binding. Oleuropein activates mtCa2+ uptake ad energy metabolism to enhance endurance and limit fatigue in young, aged but not muscle-specific MCU KO mice. Furthermore, we examined the effects of androgen depletion on intracellular Ca²# homeostasis, indicating that alterations in Ca²# signalling precede muscle atrophy and mitochondrial dysfunction. Studies in genetic animal models corroborated the role of MCU in hypogonadism-induced atrophy.

CONCLUSIONI

Mitochondrial calcium signalling controls skeletal muscle homeostasis, representing a target for intervention in conditions of diminished muscle mass and force.

B123

UNRESOLVED INFLAMMATION MARKS CHEMORESISTANCE IN MALIGNANT PLEURAL MESOTHELIOMA

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INTRODUZIONE

Malignant pleural mesothelioma (MPM) exemplifies an inflammation-driven malignancy and remains a significant therapeutic challenge owing to its marked resistance to current treatments. Inflammatory processes are believed to drive tumor progression and confer resistance to therapy. The senescence-associated secretory phenotype (SASP), induced by chemotherapy, triggers the release of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which in turn contributes to pemetrexed resistance in MPM cells in vitro.

METODI

Quantification of pro-inflammatory mediators (PIMs) and specialized pro-resolving mediators (SPMs) in pleural exudates was performed using liquid chromatography–tandem mass spectrometry. Single-cell RNA sequencing datasets were analyzed to delineate the distribution of SPM-synthesizing enzymes between mesothelial and mesothelioma cells within both tumor and uninvolved pleural tissues. LASSO regression modeling was utilized to generate a composite prognostic score (CPS) based on SPM-related gene expression profiles.

RISULTATI

Patients receiving chemotherapy demonstrated a decline in SPMs—including resolvins, protectins, and maresins—concomitant with elevated prostaglandin concentrations. Single-cell transcriptomic analysis indicated a differential allocation of SPM-synthesizing enzymes, with diminished expression observed in mesothelioma cells relative to mesothelial counterparts. The resulting five-gene CPS, derived from the lipid mediator profile, served as an independent predictor of overall survival, surpassing conventional clinical metrics.

CONCLUSIONI

Chemotherapy modifies the lipid mediator milieu of pleural exudates in MPM, notably reducing specialized pro-resolving mediators and thereby perturbing inflammatory resolution pathways. This alteration aligns with an inherent imbalance in enzyme expression between mesothelial and mesothelioma cells. Collectively, these data implicate defective resolution of inflammation in fostering a pro-tumorigenic, pro-inflammatory, and chemoresistant microenvironment in MPM, offering prognostic value.

OP001

EPIMETHEX 2.0 TOOL FOR THE INTEGRATED ANALYSIS OF DIFFERENTIALLY METHYLATED REGIONS IN CANCER

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INTRODUZIONE

Aberrant DNA methylation (methDNA) is an epigenetic hallmark of cancer driving gene dysregulation involved into immune microenvironment remodeling and treatment response. On these bases, the R-based bioinformatic tool EpiMethEx 2.0 was developed to identify differentially methylated regions (DMRs) of cancer-related genes.

METODI

EpiMethEx 2.0 was designed to perform clustering analyses of consecutive CpGs (distance $\leq$ 200 bp) based on concordant methDNA, Beta difference (median β value Tumor vs Normal), and correlation (Gene expression and methDNA of related CpGs) values in TCGA tumors (N = 33) and normal cohort, as well as combination of all the identified clusters. The translational relevance of EpiMethEx 2.0 was evaluated in a pilot multicancer cohort (N. samples = 80) by analyzing a Baculoviral IAP Repeat Containing 3 (BIRC3)-associated DMR using a bisulfite-ddPCR assay.

RISULTATI

EpiMethEx 2.0 analysis identified 147 DMRs with consistent methDNA patterns across most TCGA tumors. DLBC and LGG displayed the highest number of exclusive hypermethylated (methDNA > 0.6) CpG clusters (N > 500), whereas TGCT and OV were mainly characterized by exclusive hypomethylated (methDNA < 0.2) CG clusters (N > 100). Similarly, most of Beta difference CpG clusters were observed in DLBC and LGG, with more than 1,000 methylated (Beta diff > 0.5) or demethylated (Beta diff < -0.5) patterns. Correlation analysis retrieved 41 CpG clusters, of which 38 were negatively and 3 positively correlated with gene expression. Interestingly, intragenic CpG clusters showed a positive correlation with relative gene expression, whereas promoter CpG clusters were negatively correlated. Integrated clustering analysis of Major Histocompatibility Complex Class I genes revealed that HLA-E/F/G and TAP1 were tightly regulated by methDNA. Validation study demonstrated that the BIRC3-associated CpG cluster was exclusively hypermethylated in LGG and hypomethylated in other tumor types.

CONCLUSIONI

EpiMethEx 2.0 is a robust and reliable tool for integrative genomic analysis, enabling the identification of clinically relevant DMRs. BIRC3-associated DMR represents an LGG-specific epigenetic hallmark, showing higher diagnostic performance when analyzed as a CpG cluster rather than as a single CpG site.

OP002

DNA METHYLATION HETEROGENEITY RE-EMERGES DURING CLONAL EXPANSION OF GLIOBLASTOMA CELLS

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INTRODUZIONE

Intratumoral epigenetic heterogeneity is increasingly recognized as a major driver of tumor plasticity, adaptation, and therapeutic resistance. Although DNA methylation is generally considered a stable epigenetic mark, accumulating evidence suggests that methylation patterns may dynamically fluctuate across individual cells, generating heterogeneous combinations of methylated CpGs known as epialleles. Whether these epiallele configurations are stably inherited during tumor cell proliferation or progressively re-established during clonal expansion remains poorly understood.

METODI

To investigate DNA methylation dynamics during tumor cell proliferation, we generated putative single-cell-derived clones from U251 glioblastoma cells and expanded them through multiple proliferative stages. DNA methylation was analyzed using nanopore technologies and high-coverage bisulfite amplicon sequencing across promoters, enhancers, intronic regions, repetitive elements, and late-replicating WCGW-enriched loci. Epiallele organization was evaluated through average and single-CpG methylation, epiallele class distribution, full epiallele profiling, Shannon entropy and Jensen-Shannon distance

RISULTATI

Clonal expansion did not result in the propagation of a single dominant epiallele configuration. Instead, independently expanded glioblastoma clones progressively re-established broad and complex epiallele repertoires closely resembling those observed in the parental mixed population. While CpG-specific methylation tendencies remained largely conserved, each clone developed subtle but stable shifts in epiallele frequencies, generating clone-specific methylation equilibria. Regions displaying intermediate methylation levels exhibited the highest epiallele plasticity, whereas highly methylated or unmethylated loci maintained more constrained epiallele architectures. Notably, nanopore methylation profiling independently confirmed the presence of marked epigenetic heterogeneity among tumor clones despite their common clonal origin

CONCLUSIONI

Our findings support a model in which DNA methylation heterogeneity in tumor cells is not rigidly clonally inherited but continuously regenerated during cell proliferation within constrained genomic frameworks. These results suggest that epigenetic diversification may emerge independently of major genetic variation and represent an intrinsic property of tumor cell populations, with potential implications for cancer plasticity, evolution, and therapeutic resistance.

OP003

EXTRACELLULAR VESICLES AS BIOMARKERS IN BREAST CANCER: IMPACT OF TUMOR MODELING APPROACHES IN VITRO

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INTRODUZIONE

Extracellular vesicles (EVs) are small, spherical, non-replicating particles released by cells, involved in intercellular communication, and representing promising sources of cancer biomarkers. However, most EV studies rely on two-dimensional (2D) cultures, which poorly mimic the in vivo tumor microenvironment. Three-dimensional (3D) models may provide more physiologically relevant conditions, potentially influencing EV release and their molecular composition. This study investigated the impact of different in vitro breast cancer (BC) models on EV features using two approaches: Fourier-transform infrared spectroscopy (FT-IR) and glycan profiling of EVs.

METODI

Human BC cell lines with different levels of aggressiveness, MDA-MB-231 and MCF-7, were cultured under both 2D and 3D conditions (hanging drop method for MDA-MB-231 and ULA plates for MCF-7). EVs were isolated from conditioned media of both culture models by ultracentrifugation; the EV identity was further confirmed following MISEV2023 guidelines by Transmission Electron Microscopy, Nanoparticle Tracking Analysis, and Western blot of EV-related/ not-related markers. Finally, EVs were analyzed by FT-IR Spectroscopy and surface glycan profiling by lectin-based ELISA assays.

RISULTATI

FT-IR analysis revealed significant differences in the molecular composition of EVs derived from 2D and 3D MDA-MB-231 cultures, particularly in terms of lipid/nucleic acid ratio content, suggesting model-dependent variations in EV biological properties. In contrast, MCF-7-derived EVs showed no significant differences between the two culture systems. Glycan profiling demonstrated significant variations in surface glycan expression in EVs from both cell lines according to culture conditions. Differences were observed in galactose-, fucose-, and N-acetylglucosamine, highlighting a dynamic modulation of EV surface composition in response to the in vitro model.

CONCLUSIONI

These findings demonstrate that in vitro culture conditions can affect EV molecular composition, highlighting the importance of selecting appropriate tumor models to better reflect in vivo conditions; nevertheless, FT-IR spectroscopy and glycan profiling could be considered as promising approaches for EV-based biomarker discovery.

OP004

MECHANOBIOLOGY-GUIDED DRUG REPURPOSING IDENTIFIES BUDESONIDE AS AN INHIBITOR OF STIFFNESS-INDUCED PDAC AGGRESSIVENESS

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INTRODUZIONE

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a stiffened tumor mechanical microenvironment (TMME) that promotes malignancy and chemoresistance. Conventional rigid plastic cultures fail to replicate these physical cues. This study aims to investigate how substrate stiffness drives PDAC aggressiveness and to identify pharmacological strategies to counteract stiffness-induced mechanotransduction.

METODI

We utilized polyacrylamide (PAAm) hydrogels crosslinked at defined stiffnesses (0.2, 10 and 27 kPa) to mimic distinct mechanical states of the pancreatic microenvironment, Human commercial (PANC-1) and patient-derived PDAC cell lines were cultured on these matrices. Mechanoadaptive features, cell mechanics, YAP localization and nuclear morphometry were evaluated via immunofluorescence and confocal microscopy. Proliferation and migration were monitored through time-lapse tracking and flow cytometry - EdU assay. RNA sequencing (RNA-seq) profiling was performed to assess the transcriptional changes associated with increasing stiffness and pharmacological treatment.

RISULTATI

Increased substrate stiffness significantly enhanced cell spreading, focal adhesion size, cytoskeletal tension, proliferation, and migration, while triggering YAP nuclear translocation. Strikingly, treatment with the glucocorticoid budesonide, a FDA-approved drug used for treating asthma, effectively prevents these traits in both commercial and patient-derived lines, promoting cortical actin remodeling, restricting motility, and retaining YAP in the cytoplasm. RNA-seq profiling revealed that budesonide counteracts approximately 50% of stiffness-driven transcriptional changes, and selectively targets nuclear envelope and nucleocytoplasmic transport pathways, uncovering an unanticipated regulatory mechanism.

CONCLUSIONI

Together, these findings identify matrix stiffness as a driver of PDAC aggressiveness and reveal budesonide as a modulator of stiffness-induced mechano-transduction, supporting its repurposing as a potential anti-stiffness therapeutic approach in PDAC.

OP005

ROLE OF T3 SUPPLEMENTATION IN EARLY STAGE OF DIABETIC RETINOPATHY: EVIDENCE OF A PROTECTIVE LOCAL THYROID HORMONE ADAPTATION

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INTRODUZIONE

Diabetic retinopathy (DR) is a leading cause of vision loss. Physiologically, Triiodothyronine (T3) is a key regulator of retinal metabolism and cellular homeostasis. We previously identified a local Low T3 State (LT3S) in the retina of diabetic db/db mice, but its functional significance remains unclear. This study investigates whether LT3S represents a maladaptive condition or a protective response to hyperglycemic stress.

METODI

In vitro, human Müller cells (MIO-M1) and differentiated mouse photoreceptor precursors (661W) under high glucose (HighG) were treated with 3nM T3. Metabolism, mitochondrial network and gene expression were analyzed by Seahorse XFe96, confocal microscopy and RT-qPCR. In vivo, 10-week-old db/db mice received continuous T3 infusion (0,48 μg/Kg/die) via osmotic minipumps for 6 weeks. Retinal function was monitored longitudinally via scotopic electroretinography (scERG) and pattern electroretinography (PERG); morphological changes were assessed by image-guided optical coherence tomography (OCT) and immunofluorescence.

RISULTATI

In hyperglycemic Müller cells, abolishment of LT3S partially restored metabolic activity but increased mitochondrial fragmentation and pro-oxidant signaling. In hyperglycemic 661W cells, abolishment of LT3S triggered metabolic overactivation and marked mitochondrial disruption. In contrast, LT3S promoted a protective hypometabolic state, preserving mitochondrial integrity and limiting oxidative stress. In vivo, T3 restoration did not affect systemic metabolic parameters but significantly worsened retinal damage, with reduced ERG responses, impaired ganglion cell function, thinning of inner retinal layers, and loss of RNA-binding protein with multiple splicing-positive retinal ganglion cells (RBPMS+), associated with pro-apoptotic transcriptional changes and altered mitochondrial biogenesis.

CONCLUSIONI

LT3S represents an early, tissue-specific adaptive mechanism to hyperglycemic stress. Its reversal induces metabolic imbalance and accelerates retinal neurodegeneration, supporting a protective role of local thyroid hormone downregulation in DR.

OP006

BLOOD-BASED NEUROLOGICAL AND INFLAMMATORY BIOMARKERS PREDICT SHORT- AND LONG-TERM MORTALITY IN HOSPITALIZED OLDER ADULTS: FINDINGS FROM THE BADGER STUDY

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INTRODUZIONE

Blood-based biomarkers of neurodegeneration and neuroinflammation are emerging tools for neurological disorders, but their prognostic role in frail older adults with multimorbidity remains unclear. We evaluated whether circulating neurological and inflammatory markers are associated with mortality in a real-world population of hospitalized geriatric patients.

METODI

We analyzed plasma samples from 715 patients aged ≥65 years enrolled at INRCA within the Report-AGE study. Biomarkers measured by Simple Plex™ Ella included NfL, GFAP, total tau, UCH-L1, neurogranin, Aβ42/40 ratio, IL-6, CXCL8, lipocalin-2, MPO and α-synuclein. Values were dichotomized at median levels. Associations with 1-, 3-, 5-year and long-term mortality were assessed using logistic and Cox regression models adjusted for age, sex, frailty index, Charlson comorbidity index, renal function, RDW-CV, azotemia, NLR and albumin.

RISULTATI

Higher NfL levels were consistently associated with increased mortality across all time points. In fully adjusted models, NfL ≥67.9 pg/mL independently predicted 3-year (HR 1.94, 95% CI 1.23–3.07, p=0.005), 5-year (HR 1.59, 1.16–2.19, p=0.004) and long-term mortality (HR 1.56, 1.26–1.92, p<0.001). Elevated CXCL8 was also independently associated with 3-year (HR 1.69, 1.11–2.57), 5-year (HR 1.40, 1.04–1.89) and long-term mortality (HR 1.28, 1.05–1.57). UCH-L1 was associated with long-term mortality (HR 1.27, 1.06–1.53, p=0.011). Joint stratification of NfL and CXCL8 showed a stepwise increase in mortality risk across groups. The dual-elevation group showed the highest risk and poorest survival, confirmed by Kaplan–Meier analysis.

CONCLUSIONI

In a real-world population of hospitalized older adults, blood biomarkers of neuroaxonal damage and inflammation were independently associated with mortality beyond frailty, comorbidity and routine laboratory parameters. NfL and CXCL8 showed the strongest prognostic value. Their combined elevation identified patients at highest risk, supporting an additive interaction between neurodegenerative and inflammaging pathways. Blood-based neurological biomarkers may improve prognostic stratification in geriatric clinical practice. Supported by BADGER project: PNRR-MCNT2-2023-12378340

OP007

CUPRIZONE EXERTS MICROGLIA-MEDIATED NEUROTOXIC EFFECTS AND MODULATES AUTOPHAGY THROUGH G-QUADRUPLEXES IN NEURONAL CELLS.

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INTRODUZIONE

Demyelination is a hallmark of multiple sclerosis and several neurodegenerative diseases, in which altered central nervous system cell interactions and impaired autophagy contribute to pathology. Cuprizone (CPZ), a copper chelator widely used as an experimental demyelinating agent in animal models, has poorly defined cell-specific effects. In this study, we investigated the effects of CPZ on cells viability, microglial activation, neuronal–microglial interactions, and autophagy regulation in human neuronal (SH-SY5Y) and microglial (HMC3) cells, focusing on the role of G-quadruplex (G4) DNA structures.

METODI

CPZ effect on SH-SY5Y and HMC3 cell viability was assessed after 24 and 48h by trypan blue exclusion assay at different concentrations (1, 10, 100 μM). Microglial activation markers (CD11b, HLA-ABC, CD86, CD80, CD206 and HLA-DR), apoptosis/necrosis and G4 were evaluated by flow cytometry. Modulation of autophagy markers (BECN1 and LC3) was evaluated in SHSY5Y cells treated with CPZ (10 μM) and in the presence of quercetin (50nM) or metformin (1mM) by Western blot. Putative Quadruplex-Forming Sequences (PQFS) in the MAP1LC3A promoter were evaluated by QGRS Mapper.

RISULTATI

CPZ treatment did not directly affect neuronal or microglial cell viability, however, CM from CPZ-activated microglial cells, characterized by HLA-DR up-regulation, induced early apoptosis in SH-SY5Y cells, suggesting an indirect microglia-mediated neurotoxic effect. CPZ selectively impaired neuronal autophagy by reducing LC3II and LC3-II/LC3-I ratio without affecting BECN1 expression, no significant changes were detected in HMC3 cells. GQRS Mapper analysis identified PQFS within the LC3 promoter. Consistently, CPZ induced G4 unfolding and reduced LC3II expression in SH-SY5Y cells, similar effect was obtained with pyridostatin, a known G4 ligand. Moreover, co-treatment with neuroprotective agents, quercetin or metformin prevented CPZ-induced G4 unfolding and restored LC3 levels in SH-SY5Y cells.

CONCLUSIONI

Our data reveal a novel mechanism by which CPZ disrupts neuronal autophagy through G4 structures. Moreover, the protective effects of quercetin and metformin highlight the therapeutic potential of strategies aimed at preserving G4 stability and neuronal autophagy in demyelinating and neurodegenerative diseases.

OP008

CIRCULATING SESTRIN 2 LEVELS INCREASE WITH AGE AND ARE ASSOCIATED WITH FUNCTIONAL, BIOCHEMICAL AND INFLAMMATORY DECLINE AND HIGHER MORTALITY IN AN ITALIAN POPULATION

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INTRODUZIONE

Aging is a complex physiological process characterized by a progressive decline in bodily functions driven by alterations in key cellular mechanisms, including metabolism and inflammation. Sestrins are a family of highly conserved proteins involved in antioxidant defense and metabolic regulation, whose activity is induced by stress conditions such as oxidative stress and fasting. Among them, Sestrin2 (Sesn2) is of particular interest due to its proposed protective and anti-aging properties. Centenarians, who reach extreme longevity in good health and maintain a balanced inflammatory status, represent an important model for studying human aging.

METODI

We analyzed circulating Sesn2 levels in a cohort of 442 adults aged 29 to 111 years. Serum Sesn2 concentrations were assessed and correlated with demographic, functional, biochemical, and inflammatory parameters. Survival analysis was also performed to evaluate the relationship between Sesn2 levels and mortality. In addition, correlations with GDF15, a marker of mitochondrial stress, were investigated.

RISULTATI

Serum Sesn2 levels were significantly and positively associated with age. Higher Sesn2 concentrations correlated with worse functional, biochemical, and inflammatory profiles. Survival analysis revealed that individuals with elevated Sesn2 levels had significantly reduced survival compared to those with lower levels. Sesn2 also showed a positive correlation with GDF15. Furthermore, frail individuals exhibited higher Sesn2 levels, suggesting a link between Sesn2 and frailty status.

CONCLUSIONI

Overall, our findings indicate that increased circulating Sesn2 levels with aging may reflect an adaptive response to rising cellular stress. However, elevated Sesn2 is also associated with frailty, poorer health status, and reduced survival, suggesting its potential role as a biomarker of biological aging.

OP009

SIMVASTATIN PROTECTS HUMAN CARDIOMYOCYTES AGAINST DOXORUBICIN-INDUCED TOXICITY BY MODULATING CELL CYCLE PROTEINS AND APOPTOTIC PATHWAYS

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INTRODUZIONE

Doxorubicin (Doxo) is a widely used chemotherapeutic agent whose clinical application is limited by cardiotoxicity. Although adult cardiomyocytes are terminally differentiated, Doxo disrupts cell cycle-related pathways, inducing S-phase reentry via CDK2 activation, suggesting this kinase regulates cardiac stress responses. Several studies have reported that cell cycle proteins are regulators of cell death: CDK2 enhances Bim expression in Doxo-induced apoptosis, and the CyclinB1-CDK1 complex regulates apoptotic signaling. Additionally, the p21 gene is involved in myocardial damage. Statins, including Simvastatin (Sim), modulate cell cycle progression in cancer cells, but evidence on cardiomyocytes is limited.

METODI

Human Cardiomyocyte (HCM) lines were pre-treated with Sim (10μM) for 4 h and co-exposed to Sim and Doxo (1μM) for 20 h. Real-time qPCR evaluated CDK1, CDK2, Cyclin A, Cyclin B1, ErbB2, p21, and TβRII expression. Flow cytometry (Cell Quest/ModFit) analyzed the cell cycle. PARP, Caspase-7, and Bax expression was evaluated by Western blot. One-way ANOVA followed by Tukey's test was used ($p < 0.05$ was considered statistically significant).

RISULTATI

In our in vitro model, Sim co-treatment significantly reduced Doxo-induced gene overexpression of CDK1 ($p < 0.001$), CDK2 ($p < 0.001$), Cyclin A ($p < 0.005$), and Cyclin B1 ($p < 0.001$). Doxo significantly increased cells in S phase ($p < 0.001$), an effect reversed by Sim ($p < 0.01$). Western blot showed that Sim reduced PARP, Caspase-7, and Bax expression, supporting its protective role against Doxo-induced cell death. Furthermore, Sim enhanced ErbB2 expression ($p < 0.05$), involved in cardiac development and stress response, and significantly reduced Doxo-induced overexpression of p21 ($p < 0.001$) and TßRII ($p < 0.005$), both linked to cardiac fibrosis.

CONCLUSIONI

Our results suggest that Sim reduces Doxo-induced cardiac death by modulating CDKs and cyclins, thereby affecting the cell cycle. This is consistent with emerging evidence indicating that cell cycle proteins play an unconventional role in regulating cell death.

OP010

EPIGENETIC STRATIFICATION IN EGFR AND KRAS NSCLC PATIENTS: ROLE OF ABERRANT METHYLATION OF CD274/PD-L1 GENE

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INTRODUZIONE

Non-small cell lung cancer (NSCLC) is a biologically complex and heterogeneous malignancy characterized by distinct oncogenic drivers, including epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations. Although progress has been made in targeting these subgroups, clinical outcomes remain variable due to resistance, tumor relapse and therapy sensitivity. Epigenetic reprogramming has emerged as a key feature involved in resistance, clonal tumor evolution and prognosis.

METODI

Herein, we report preliminary results of a large-scale data-driven study investigating the clinical significance of epigenetic alterations in NSCLC. DNA methylation profiling of differentially methylated regions (DMRs) within the Programmed Death-Ligand 1 (CD274/PD-L1) gene was assessed in NSCLC cell lines and integrated with multi-omics data from an independent NSCLC cohort of The Cancer Genome Atlas (TCGA, n=1188). The analysis included lung adenocarcinoma (LUAD, n=617) and lung squamous cell carcinoma (LUSC, n=571), across disease stages and stratified by EGFR/KRAS status and smoking history.

RISULTATI

In NSCLC cell lines, considerable variability in methylation levels was observed across promoter regions of CD274/PD-L1. Treatment with a demethylating agent showed an inverse correlation between promoter methylation and CD274/PD-L1 mRNA levels ($p=0.002$, t-test).

Analysis of the independent TCGA dataset confirmed the significant effect of CD274/PD-L1 methylation on transcript and protein levels. Correlations between methylation, mRNA and protein were heterogeneous and dependent on histology and CpG site position. Distinct patterns were observed according to CpG localization within promoter, intragenic regions or North-South shores (N/S). In stage IV LUAD with EGFR mutations, promoter methylation was inversely correlated with transcriptional activity, whereas N/S CpGs showed a direct positive correlation. A heterogeneous relationship between KRAS mutational status and CD274/PD-L1 methylation was observed in LUAD for extra-promoter CpG sites, while no such correlation was found in LUSC.

CONCLUSIONI

Although the analysis was not performed in cohorts treated with EGFR tyrosine kinase inhibitors (TKIs) and lacks longitudinal data on treatment responsiveness, these findings support the hypothesis that aberrant DNA methylation is a characteristic feature of EGFR-mutated tumors and may influence tumor behavior, therapeutic response and clinical outcome.

OP011

SOMATIC FOCALLY AMPLIFIED SUPER-ENHANCERS AS NOVEL ONCOGENIC DRIVER IN NEUROBLASTOMA

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INTRODUZIONE

Neuroblastoma (NB) is a pediatric cancer with poor prognosis, often driven by somatic focal amplifications (FAs) that enhance oncogene expression, including MYCN and ALK. Emerging evidence suggests that non-coding FAs can involve super-enhancers (SEs), controlling cell identity and may contributing to tumor development. However, the specific role of SEs within focal amplifications (FA-SEs) in neuroblastoma remains poorly understood. In our study hypothesize that FA-SEs drive tumorigenesis by upregulating critical oncogenes and influencing disease progression. Our goal is to identify neuroblastoma-specific FA-SEs and clarify their impact on tumor biology and clinical outcomes.

METODI

To identify NB-specific FA-SEs, we integrated WGS and public NB-specific SE datasets. FA-SE targets were predicted using capture Hi-C, RNA-seq, and H3K27ac ChIP-seq data, and validated by ChIP-seq and RT-PCR in NBL-S and SK-N-AS cells after treatment with epigenetic-modulating compounds. Enhancer silencing by viral CRISPR-dCas9-KRAB-MeCP2 was further confirmed by PCR, while functional effects were assessed by MTT, scratch-wound, and colony formation assays.

RISULTATI

Analysis of WGS data from 136 NB patients uncovered four FA-SEs at 11q13.3 and 17q25.3, strongly associated with high-risk clinical and prognostic features, and subsequently validated in an independent cohort of 180 patients. Integrative multi-omics analyses combining capture Hi-C, RNA-seq, and H3K27ac ChIP-seq datasets identified oncogenic FA-SE regulatory networks involving key cancer-related genes, including CCND1, FGF19, MYEOV, TPCN2, BIRC5, and TK1. Pharmacological perturbation of enhancer-associated chromatin states using epi-drugs targeting H3K27ac and H3K4me1 dynamics induced extensive transcriptional rewiring of FA-SE target genes, supporting their epigenetic dependency. Moreover, CRISPR-dCas9-KRAB-MeCP2-mediated silencing of FA-SEs in NBL-S and SK-N-AS cells markedly reduced proliferation, migration, and clonogenic potential, functionally confirming the oncogenic role of these regulatory elements.

CONCLUSIONI

Our findings demonstrate that somatic focal amplifications reshaping super-enhancer landscapes promote oncogenic transcriptional programs, driving NB progression and aggressive clinical behavior. These FA-SEs not only represent robust biomarkers for patient stratification and prognostic assessment, but also uncover actionable epigenetic vulnerabilities, highlighting their potential as innovative therapeutic targets in neuroblastoma.

OP012

THE ONCOSTATIN M/OSMRβ AXIS SHAPES THE TUMOR MICROENVIRONMENT AND ENHANCES INVASIVENESS IN INTRAHEPATIC CHOLANGIOCARCINOMA

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INTRODUZIONE

Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver malignancy and is characterized by high aggressiveness and limited therapeutic options. Increasing evidence highlights the crucial role of the tumor microenvironment (TME) in driving tumor progression and therapy resistance. Oncostatin M (OSM), a pleiotropic cytokine belonging to the IL-6 family, has emerged as a potential mediator of tumor–stroma communication through activation of the JAK/STAT3 signaling pathway. This study aimed to investigate the role of the OSM/OSMRβ axis in iCCA progression and in the crosstalk between tumor cells and the surrounding microenvironment.

METODI

OSM signaling was investigated in human iCCA models, including 2D and 3D cultures, patient-derived organoids (PDOs), stromal cells, and clinical specimens. Its activity was modulated by OSM stimulation, OSMRβ silencing, or ruxolitinib treatment, and evaluated through functional, molecular, transcriptomic, and immunohistochemical analyses.

RISULTATI

OSMRβ was constitutively expressed in iCCA cell lines, patient-derived organoids (PDOs), and tumor tissues. OSM stimulation activated multiple signaling pathways (STAT3, ERK1/2, AKT, JNK, and STAT5), inducing epithelial–mesenchymal transition (EMT) and promoting migration, invasiveness, angiogenesis, and stemness programs. OSM also enhanced cancer-associated fibroblast (CAF) recruitment and macrophage polarization toward a tumor-supportive phenotype, contributing to extensive remodeling of the tumor microenvironment. Transcriptomic analyses confirmed enrichment of mesenchymal pathways and suppression of immune-related programs. Mechanistically, OSMRβ silencing or pharmacological inhibition with ruxolitinib significantly attenuated OSM-induced signaling, EMT, migration, angiogenic responses, stemness-associated markers, CAF recruitment, and macrophage reprogramming. In human samples, circulating OSM levels were decreased in iCCA patients and associated with better survival, whereas tumor OSM/OSMRβ expression was increased and correlated with dedifferentiation and immunosuppressive markers, supporting a role for OSM signaling in tumor progression and immune microenvironment remodeling.

CONCLUSIONI

The OSM/OSMRβ axis drives iCCA progression through JAK/STAT signaling and represents a promising biomarker and therapeutic target, given its association with tumor aggressiveness and its susceptibility to pharmacological inhibition.

OP013

METABOLIC DOMINANCE OF GLYCOLYSIS OVER LIPID METABOLISM SHAPES OSTEOSARCOMA HETEROGENEITY AND DRUG RESPONSE

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INTRODUZIONE

Osteosarcoma shows marked heterogeneity, but the metabolic programs driving progression and therapy response remain unclear. The roles of glycolysis and lipid metabolism and their integration with genomic and microenvironmental features are not fully defined.

METODI

Single-cell RNA-seq data from osteosarcoma and normal bone were integrated with bulk transcriptomic cohorts. Analyses included CNV inference, pseudotime, gene set enrichment, ligand–receptor modeling, and WGCNA. Metabolic dependencies were validated in cell lines using LDHA and FASN inhibition combined with transcriptomic, proteomic, metabolomic, and lipidomic profiling. Clinical relevance was assessed through survival analysis and drug response prediction.

RISULTATI

Single-cell analysis identified CNV-defined malignant and non-malignant populations sharing an osteolineage trajectory. Aneuploid cells were enriched in proliferative states and showed increased glycolytic activity. Across datasets, glycolysis was consistently enriched, whereas lipid metabolism showed weak effects. Bulk RNA-seq confirmed glycolysis activation in osteosarcoma, associated with poor survival, proliferation programs, and immune remodeling. Fatty acid metabolism lacked prognostic value. WGCNA identified a glycolysis-associated module linked to tumor progression, highlighting LDHA as a key driver, while FASN showed limited relevance. Functional assays demonstrated selective dependency on glycolysis in glycolysis-high models, where LDHA inhibition impaired proliferation and metabolic function, whereas lipid targeting had weaker effects. Multi-omics analyses confirmed glycolytic rewiring with lactate accumulation and coordinated redox and nucleotide metabolism changes. Lipid metabolism showed context-dependent and less consistent alterations.

CONCLUSIONI

Osteosarcoma heterogeneity is primarily driven by a glycolysis-centered program linking genomic instability with tumor progression, immune remodeling, and therapeutic response, enabling clinically relevant metabolic stratification. Lipid metabolism plays a secondary, context-dependent role. These findings identify glycolysis as a central metabolic vulnerability and a framework for future stratification and precision therapy in osteosarcoma.

OP014

AMBRA1 REGULATES SONIC HEDGEHOG SIGNALING DURING CEREBELLAR DEVELOPMENT AND PROMOTES SHH-MEDULLOBLASTOMA

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INTRODUZIONE

AMBRA1 (Autophagy and beclin 1 regulator 1) is a scaffold protein crucial for early nervous system development. While primarily recognized as a tumor suppressor, its context-dependent pro-tumoral functions are increasingly emerging. Prompted by our finding that AMBRA1 protein is significantly abundant in Sonic Hedgehog medulloblastoma (SHH-MB), a highly aggressive pediatric brain tumor, here we unveil its role in SHH-MB pathogenesis and physiological cerebellar development.

METODI

AMBRA1 protein and mRNA expression levels were analyzed in an international multi-omic cohort of human MB samples. Proteomic analysis of the tumor suppressor REN was performed to identify novel partners. Bulk and single-cell RNA sequencing identified downstream SHH target genes regulated by AMBRA1. Functional validation was conducted in vitro and in vivo using murine models and patient-derived xenografts (PDXs) of HH-MB subjected to shRNA-mediated AMBRA1 depletion, alone or combined with FDA-approved SMO inhibitors. In vivo postnatal electroporation in mice and generation of Math-cre/Ambra1$^{fl/fl}$ mouse model evaluated AMBRA1 during cerebellar development.

RISULTATI

High AMBRA1 protein expression, but not mRNA, selectively correlates with worse overall survival in SHH-MB patients. Mechanis¬tically, AMBRA1 binds the E3 ligase βTrCP, protecting the transcription factor GLI1 from ubiquitination and proteasomal degradation via an autophagy-independent mechanism. Upstream, AMBRA1 protein accumulation is restrained by the tumor suppressor REN, a Cul3-E3 ubiquitin ligase frequently lost in SHH-MB, which targets AMBRA1 for degradation. Preclinically, AMBRA1 silencing suppresses tumor growth in both murine allografts and human PDX models. Strikingly, combining AMBRA1 depletion with SHH inhibitors exertes a powerful synergistic effect, completely abolishing tumor progression in vivo. In developing cerebella, AMBRA1 genetic depletion in mice impairs granule neuron progenitor (GNP) proliferation.

CONCLUSIONI

Our findings identify the AMBRA1/βTrCP/REN axis as a critical molecular hub sustaining SHH signaling. Unveiling AMBRA1 as an oncogenic driver in medulloblastoma provides actionable insights into brain tumor biology, highlighting AMBRA1 as a promising therapeutic target to overcome drug resistance in SHH-dependent malignancies.

OP015

FROM PATIENT TO PREDICTION, AUTOLOGOUS INFLAMED SYNOVIUM-ON-CHIP MODELS TO FORECAST THERAPEUTIC RESPONSE IN RHEUMATOID ARTHRITIS

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INTRODUZIONE

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting joints, with a global prevalence of ~0.5–1%. Despite multiple therapeutic options, over 40% of patients still experience long-term disability due to the lack of predictive tools for treatment response. To address this unmet need, we developed an autologous patient-derived synovium-on-chip (SoC) platform enabling personalized trial-on-chip approaches for therapy prediction in RA.

METODI

A 3D microfluidic SoC platform was engineered to host autologous leukocyte-infiltrated synovial microtissues generated from RA biopsies. Fibroblast-like synoviocytes (FLSs) and peripheral blood mononuclear cells (PBMCs) were isolated from methotrexate (MTX) responders (R) and inadequate responders (IR). Cells were encapsulated in 5% GelMA and cultured under continuous perfusion (40 μL/h). After inflammatory stimulation with IL-6, IL-1β and TNF-α, MTX (1 μM) was administered for ~48 h. Readouts included viability, lubricin secretion, cytokine profiling (>50 cytokines), extracellular vesicle (EV) analysis and single-cell RNA sequencing.

RISULTATI

The SoC supported the formation of viable 3D synovial microtissues reproducing key RA features. RA-FLS viability exceeded 80% in monoculture and 60% in co-culture with PBMCs. In FLS-only SoC, MTX induced higher log2FC (MTX/CTR) viability changes in IR than in R, suggesting differential drug sensitivity. Lubricin secretion significantly increased in R compared with IR ($p<0.05$). Cytokine profiling revealed clear separation between R and IR by principal component analysis (PCA), with MMP-2, IL-28A, IL-34, IL-22 and IL-26 as major contributors. EV secretion also differed between groups, showing changes in size and concentration. Single-cell RNA-seq identified a distinct FLS subpopulation enriched in MTX-IR samples, potentially associated with treatment resistance. In FLS–PBMC co-culture SoC, inflammatory stimulation induced significantly lower cytokine levels in R than in IR ($p<0.05$), further supporting patient-specific inflammatory signatures.

CONCLUSIONI

This autologous human SoC reproduces key aspects of RA pathophysiology and therapeutic response, enabling rapid patient-specific drug testing while reducing reliance on animal models. Beyond therapy prediction, the platform provides a versatile tool for studying RA heterogeneity, inflammatory mechanisms and resistance-associated cellular states, supporting precision medicine in RA.

OP016

GLUTAMINE DERIVED FROM HUMAN MACROPHAGES IS NEEDED FOR IFN GAMMA SECRETION BY CD8+ LYMPHOCYTES.

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INTRODUZIONE

Functional activation of $CD8^+$ T-cells requires cooperation by M1-polarized macrophages through antigen presentation, co-stimulation and cytokine secretion. At the same time, the interaction may also involve a metabolic crosstalk between the two cells. Although L-glutamine (Gln) is needed for the activity of $CD8^+$ lymphocytes, what is the source of the amino acid in the immune microenvironment is not known. We found that M1-polarized THP1 macrophage-like cells boost Gln synthesis by GLUL and its extrusion through the transporter SNAT5 (manuscript under revision). Here we show that the same effects occur in human monocyte-derived macrophages (MDM).

METODI

Monocytes and lymphocytes were obtained through immunomagnetic purification of buffy coat from healthy donors. M1 polarization of MDM was achieved with LPS/IFNγ, while functional activation of co-cultures of lymphocytes/ macrophages was obtained exposing MDM to Bifidobacterium bifidum and assessed through IFNγ secretion. The expression of Gln-related enzymes and transporters was assessed with RT-PCR, WB and IF. Transport activity was determined through the influx of [^{3}H]-Gln.

RISULTATI

M1 polarization markedly stimulated SNAT5 and GLUL expression in MDM with the transporter mainly localized at the plasma membrane. Gln transport was also increased, and the stimulation was abolished by the SNAT5 inhibitors His and GluγHA. SNAT5 and GLUL expression were also higher in MDM, co-cultured with $CD8^+$ T-cells and exposed to bacteria, than in control, not exposed co-cultures. Bacterial exposure also caused the massive increase of IFNγ secretion that required the presence of both cell types. Cytokine production was prevented by the GLUL-inhibitor MSO in the absence but not in the presence of physiological levels of extracellular Gln. However, MSO did not prevent SNAT5 or GLUL induction.

CONCLUSIONI

These data suggest that Gln availability is required for IFNγ secretion by MDM-stimulated $CD8^+$ lymphocytes. If the amino acid is not supplemented in the extracellular medium, it can be provided by MDM intracellular synthesis and secretion. Thus, in the context of inflammatory microenvironment, a metabolic cross talk between MDM and $CD8^+$ lymphocytes exists to support and, possibly, to modulate T-cell activity.

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OP017

FFPE-OPEN-ST: WHOLE-TRANSCRIPTOME SPATIAL TRANSCRIPTOMICS UNLOCKS THE POTENTIAL OF ARCHIVAL CLINICAL SAMPLES

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INTRODUZIONE

Formalin-fixed paraffin-embedded (FFPE) tissue blocks represent the gold-standard methods for preservation of tissue samples in clinical practice. Applying spatial transcriptomics (ST) to FFPE specimens provides unprecedented access to a vast repository of biological samples that can be useful for medical research including retrospective studies, given the long-term follow-up information, and documented therapeutic outcomes. However, transcriptome-wide ST profiling of FFPE tissues remains challenging due to formalin-induced crosslinking, which severely compromises RNA integrity and accessibility. FFPE-compatible ST technologies remain limited, and commercially available options rely mostly on probe-based approaches, which confine analyses to predefined gene panels. To overcome these limitations, we developed FFPE-Open-ST, a high-resolution whole-transcriptome platform for FFPE tissues with an integrated computational pipeline.

METODI

To compare FFPE and paired FF ST datasets, we first assessed the correlation between the protein-coding genes captured. Then, spatial domains were identified using Squidpy in Python and annotated based on marker genes. Pearson correlation was computed between shared cell types across FFPE and FF samples. To assess robustness and applicability to long-term archived specimens, we applied the method to FFPE mouse brain samples preserved for up to 6.5 years.

RISULTATI

FFPE Open-ST generates high-quality spatial transcriptomic data comparable to standard Open-ST on FF samples. Spatial expression patterns of canonical marker genes were highly consistent between FF and FFPE samples, both in terms of expression levels and spatial localization. The same cell types were identified in the FFPE and FF samples. Furthermore, in mouse samples stored for up to 6.5 years, we successfully identified the expected major cell populations and cell type-specific lncRNAs, with spatial patterns that remained stable over time.

CONCLUSIONI

FFPE-Open-ST enables high-resolution, unbiased spatial transcriptomics profiling of archival samples. This study lays the groundwork for broader studies on archived clinical samples with the aim to identify biomarkers and investigate mechanisms of therapy resistance.

OP018

SNOWBALL EFFECT: A SYNERGISTIC NANOTECHNOLOGY PLATFORM FOR PRECISION RADIOSENSITIZATION OF TRIPLE-NEGATIVE BREAST CANCER

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INTRODUZIONE

Metastatic tumors are often resistant to conventional therapies, including radiotherapy. Radiotherapy works by damaging cancer cell DNA through ionizing radiation, but its efficacy is frequently reduced by tumor hypoxia, a condition characterized by low oxygen levels that limits the production of reactive oxygen species (ROS) required to enhance radiation-induced damage. Consequently, hypoxic tumor cells can survive treatment, contributing to metastasis, recurrence, and therapeutic failure. Although gold nanoparticles (AuNPs) are recognized as effective radiotherapy enhancers because of their high radiation absorption capacity, their clinical use is limited by rapid systemic clearance and the requirement for high, potentially toxic, doses.

METODI

he therapeutic efficacy and biological effects of the platform were evaluated using colony formation assays to assess radiosensitivity and cell survival, Western blot analysis to investigate the expression of proteins associated with stress response and cell death pathways, and real-time PCR to analyze gene expression changes related to hypoxia, oxidative stress, and tumor response. Inductively coupled plasma mass spectrometry (ICP-MS) was employed to quantify gold nanoparticle uptake, biodistribution, and accumulation within cells and tumor tissues.

RISULTATI

Infrared activation of the gold nanorods generated localized hyperthermia, damaging tumor cells and promoting the release of CNC-Au-Glc radiosensitizing nanoparticles. The ECFC-mediated delivery system enabled enhanced nanoparticle accumulation in tumors, including hypoxic regions that are typically difficult to treat. The sequential combination of photothermal therapy followed by X-ray irradiation amplified ROS production and radiation-induced damage, potentially overcoming tumor radioresistance while reducing the need for high systemic nanoparticle doses.

CONCLUSIONI

This strategy presents a multifunctional precision radiotherapy platform that integrates photothermal therapy, radiosensitization, and cell-mediated nanoparticle delivery to target resistant metastatic tumors. By combining ECFC-based tumor homing with tumor-targeted AuNP constructs, the approach aims to improve nanoparticle retention, enhance ROS-mediated radiation damage, overcome hypoxia-associated radioresistance, and reduce off-target toxicity. The coordinated therapeutic cascade represents a promising avenue for more effective and selective cancer treatment.

OP019

TARGETING CIRCADIAN DYSREGULATION AND AIRWAY INFLAMMATION IN CYSTIC FIBROSIS USING INHALABLE ILOPROST NANO-IN-MICRO FORMULATIONS: AN EX VIVO AND EXOME-BASED STUDY.

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INTRODUZIONE

Circadian clock genes regulate inflammatory responses in lung diseases, but their role in cystic fibrosis (CF) remains unclear. This study investigated the expression of clock genes in human nasal epithelial cells (hNEC) derived from CF patients and evaluated the modulatory effects of inhalable Nano-in-Micro (NiM) formulations carrying Iloprost (ILO). In addition, whole-exome sequencing (WES) was performed to identify genetic variants potentially involved in inflammation and circadian regulation.

METODI

hNEC, obtained by nasal brushing from 10 CF patients carrying homozygous or compound heterozygous F508del mutations and cultured under air-liquid interface (ALI) conditions, were exposed to lipopolysaccharide (LPS) from Pseudomonas aeruginosa alone or in combination with free ILO and ILO-loaded Nano-in-Micro formulations (NiM-ILO and NiM-PEG-ILO). The expression of clock genes (BMAL1, CLOCK and NR1D1) was assessed by quantitative real-time PCR. WES and bioinformatic analyses were performed on freshly isolated hNEC to find genomic variants in circadian and inflammatory genes.

RISULTATI

LPS reduced the expression of CLOCK, BMAL1 and NR1D1 in hNEC ALI cultures. Treatment with ILO partially restored clock gene expression, whereas NiM formulations, particularly NiM-PEG-ILO, showed the strongest recovery effect on all circadian genes analyzed. WES identified variants in genes associated with circadian and inflammatory pathways, including CLOCK, PER1 and NFKB2 in all analyzed subjects. According to American College of Medical Genetics and Genomics (ACMG) criteria, these variants, classified as benign, may act as potential modifiers of inflammatory and epithelial responses in CF.

CONCLUSIONI

Circadian clock genes, dysregulated in CF airway epithelial cells under inflammatory conditions, may be modulated by ILO-based NiM formulations. Identified variants in circadian and inflammatory pathways may contribute to variability in CF inflammatory responses, supporting their potential role as therapeutic targets.

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OP020

TARGETING YAP/TAZ TO OVERCOME DRUG RESISTANCE IN MELANOMA CELLS

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INTRODUZIONE

Melanoma is an aggressive skin cancer. Although surgical excision cures most primary tumors, many patients develop metastases requiring systemic therapy. Acquired resistance after chronic exposure to targeted agents remains the major clinical limit. Elevated YAP/TAZ signaling has been reported in multiple tumors, including melanoma, and correlates with aggressive, drug-resistant phenotypes. Here, we characterized melanoma cells with acquired resistance to combined BRAF/MEK inhibition and evaluated YAP/TAZ as a target to restore drug sensitivity.

METODI

Human resistant 501Mel melanoma cells were obtained by continuous exposure to dabrafenib + trametinib (D+T) for ≥3 months. The resistant phenotype was characterized by flow cytometry for cell cycle, colony-forming assay, and 3D spheroid growth. We assessed anoikis resistance and analyzed key survival/proliferation markers by Western blot. YAP/TAZ nuclear translocation was evaluated by immunofluorescence. The effect of the YAP inhibitor CA3, alone and with D+T, was determined by MTT, live/dead staining, colony-forming assay, and Western blot.

RISULTATI

Upon D+T exposure, resistant 501Mel cells retained clonogenicity and 3D spheroid growth with no significant changes in cell cycle distribution. Increased N-cadherin suggested an EMT-like program in resistant cells. D+T treatment suppressed pERK in both parental and resistant cells, but resistant cells showed a compensatory increase in pS6 levels; further, resistant cells displayed constitutive YAP/TAZ nuclear translocation. YAP inhibition with CA3 enhanced D+T cytotoxicity, reducing cell viability and clonogenicity while suppressing anoikis resistance. Mechanistically, YAP/TAZ inhibition triggered ferroptosis in resistant cells, overcoming D+T tolerance.

CONCLUSIONI

These findings identify YAP/TAZ as a key mediator of melanoma drug resistance and support its targeting as a promising strategy to enhance the efficacy of BRAF/MEK inhibitors and overcome acquired resistance associated with chronic drug exposure.

OP021

NOVEL INSIGHTS INTO THE PATHOGENETIC MECHANISMS OF CANCER RESISTANCE AGAINST TOPOTECAN: THE ROLE OF GLUTATHIONE TRANSFERASE OMEGA 1-1.

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INTRODUZIONE

Glutathione transferase omega 1-1 (GSTO1-1) is an enzyme highly expressed in a number of cancers with both reductase and thioltransferase activity. These atypical properties allow GSTO1-1 to modulate the redox state of both exogenous and endogenous substrates, including factors involved in cell signaling.
Previous results obtained in our laboratories have demonstrated that GSTO1-1 modulates the cellular response against cisplatin and arsenic trioxide through the regulation of key intracellular signaling pathways involved in cell survival and proliferation. Interestingly, some preliminary data obtained with Topotecan, i.e. a drug with a mechanism of action other than cisplatin and arsenic trioxide, seem to suggest a similar interpretation. The aim of this study was thus to further investigate the GSTO1-1-dependent resistance of cancer cells against Topotecan.

METODI

Two cell lines with different levels of GSTO1-1 were obtained from the cervical carcinoma derived HeLa cell line, i.e. the HeLa GSTO1-1 stable transfected (HeLaGSTO1+) cell line and the HeLa CRISPR/Cas9 KO (HeLaGSTO1-) cell line. The levels of apoptosis were evaluated by Hoechst staining and MAPKs activation by immunoblot. Modifications of GSTO1-1 localization were evaluated by IHF and cell migration by the scratch assay.

RISULTATI

The survival of HeLaGSTO1+ cells was higher at all concentrations and time points selected, confirming that GSTO1-1 expression can efficiently protect cells against Topotecan toxicity. Indeed, the activation of prosurvival pathways (Akt and ERK1/2) and a lower number of apoptotic cells were detectable in the more resistant HeLaGSTO1+ cells. The scratch assay also revealed a greater migratory capacity for HeLaGSTO1+ cells as compared with HeLaGSTO1- cells.

CONCLUSIONI

Our results confirm and further support the conclusion that GSTO1-1-dependent resistance of cancer cells against anticancer drugs could depend on the modulation of common pro-survival pathways, rather than the detoxification of specific compounds. These observations once again highlight the distinctive features of GSTO1-1 which differs significantly from other glutathione transferases in both structural and functional properties.

OP022

SUCCINATE-GENERATED REDOX POTENTIAL ENHANCES THE EFFICACY OF CHEMOSENSITIZERS IN OVERCOMING CANCER DRUG RESISTANCE

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INTRODUZIONE

Mitochondrial electron transport can be partly maintained through non-canonical pathways when the electron transport chain (ETC) is inhibited. Activation of these pathways requires available reducing equivalents and redox mediators. We examined the effects of three compounds - quercetin, a redox-active flavonoid; methylene blue (MB), an electron donor/acceptor; and dimethysuccinate (DS), a reducing substrate - on respiration in two cellular models with distinct metabolic profiles: HLE-neo parental cells, which have very low succinate levels, and sorafenib-resistant HLE-LPAR6 cells, which have high succinate levels.

METODI

High-resolution respirometry was performed on intact cells after inhibiting complexes I and III with rotenone and antimycin A, respectively. Succinate intracellular levels were measured by NMR-Based Metabolomics, and the cells were subsequently treated with MB, either alone or in combination with quercetin. The contribution of reducing equivalents was assessed through DS supplementation or complex II inhibition.

RISULTATI

ETC inhibition suppressed oxygen consumption in both cell types. Adding MB restored respiration, showing its ability to sustain electron transfer in the shut-down ETC. The addition of quercetin resulted in a striking difference, as MB failed to restore respiration in succinate-deficient HLE-neo cells, but remained effective in LPAR6 cells. DS supplementation restored mitochondrial bioenergetics in quercetin-treated HLE-neo, indicating that reducing equivalent availability, not ETC defects, is the limiting factor. Conversely, complex II inhibition did not prevent MB-driven respiration in HLE-LPAR6 cells. Uncoupling with FCCP reduced oxygen consumption following MB treatment in both models, indicating a partial dependence on mitochondrial membrane potential.

CONCLUSIONI

Quercetin, MB, and succinate interact to set a redox threshold, where quercetin limits electron availability, MB acts as an electron shuttle that requires sufficient reducing equivalents, and succinate boosts intracellular reducing power. Resistant cells maintain a redox environment that supports MB-driven alternative respiration, even under constrained conditions. This reveals a metabolic vulnerability that could be exploited therapeutically.

OP023

ESTABLISHING METTL16 AS A KEY REGULATOR OF GBM CARCINOGENESIS

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INTRODUZIONE

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor in adults, characterized by rapid proliferation, high heterogeneity, and poor prognosis, with a median survival of ~15 months despite standard therapy. Increasing evidence highlights the role of N6-methyladenosine (m6A) RNA modification in cancer biology. While METTL3 has been extensively studied, the contribution of the m6A writer METTL16 to GBM remains largely unexplored.
This study aims to define the role of METTL16 in GBM progression, evaluate its impact on tumor cell survival and proliferation, and investigate the underlying molecular mechanisms, assessing its potential as a therapeutic target.

METODI

METTL16 expression was analyzed using TCGA, GTEx, and
CPTAC datasets and validated in GBM cell lines (U87, T98G) and patient-derived glioma stem-like cells.
Loss-of-function studies were performed via shRNA-mediated knockdown, and pharmacological inhibition targeted METTL16 enzymatic activity. Functional assays included proliferation, clonogenicity, apoptosis (PI staining, PARP cleavage), and cytotoxicity.
Transcriptomic changes were assessed by RNA-seq and Gene Set Enrichment Analysis, followed by RT-qPCR and immunoblot validation. In addition, m6A RNA immunoprecipitation sequencing (MeRIP-seq) was used to identify methylated transcripts and distinguish direct METTL16-dependent regulation from indirect transcriptional effects.

RISULTATI

METTL16 is significantly overexpressed in GBM tissues and cell models; its depletion reduces proliferation, clonogenicity, and metabolic activity while inducing apoptosis, effects recapitulated by pharmacological inhibition. Transcriptomic analysis revealed downregulation of mTOR, MYC, and E2F signaling pathways. Mechanistically, METTL16 targeting disrupts the mTOR/S6K axis, and mTOR expression positively correlates with METTL16, suggesting a functional link.

CONCLUSIONI

Overall, METTL16 emerges as a key regulator of GBM cell survival and proliferation, and integration of RNA-seq with MeRIP-seq enables identification of direct epitranscriptomic targets, supporting METTL16 as a promising therapeutic strategy warranting further validation.

OP024

HYPOXIA-DRIVEN EPHA2 TARGETING ENABLES A40S–MIR-340-5P DELIVERY TO DISRUPT GLIOBLASTOMA STEM CELLS

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INTRODUZIONE

Glioblastoma multiforme (GBM) is a highly aggressive brain tumour, characterized by recurrence and resistance to conventional therapies. Glioma stem cells (GSCs), particularly enriched in hypoxic niches, play a central role in tumour progression and adapt to hostile microenvironments, including low oxygen and acidic conditions. Carbonic anhydrase IX (CA9) is overexpressed in GSCs and upregulated under hypoxia via HIF-1A. We identified a regulatory mechanism in which miR-340-5p, a tumour-suppressor microRNA downregulated in GBM, directly targets CA9, as predicted by bioinformatic analyses and confirmed through experimental validation. Furthermore, hypoxic conditions suppress miR-340-5p through HIF-1A, which directly binds the miR-340 promoter, as revealed by chromatin immunoprecipitation (ChIP) assays. Building on these findings, we developed an innovative therapeutic strategy to target hypoxic niches in GBM using a pioneering aptamer–microRNA platform.

METODI

We generated an A40s–miR-340-5p chimeric construct in which the A40s RNA aptamer serves as a carrier for the selective delivery of miR-340-5p into GSCs. A40s preferentially binds GSCs over differentiated GBM cells through recognition of the erythropoietin-producing hepatocellular receptor A2 (EphA2) and is rapidly internalized. To investigate the role of EphA2 in hypoxia-driven targeting, we generated EphA2-knockout U251-MG cells using the CRISPR/Cas9 system.

RISULTATI

Following efficient uptake by GSCs, the A40s–miR-340-5p chimera downregulates CA9, inhibits stem-like features, reduces cell growth and promotes apoptosis. In addition, treatment decreases microenvironment acidification and favours a shift toward normoxia-like conditions. EphA2-knockout cell line was validated at protein, transcript, and cell-surface levels. While U251-MG cells displayed minimal binding of A40s-miR-340-5p due to low basal EphA2 expression, hypoxic exposure increased EphA2 levels and enhanced aptamer binding. In contrast, EphA2-knockout cells failed to acquire binding capacity under hypoxic conditions, demonstrating that hypoxia-dependent targeting is strictly mediated by EphA2 upregulation.

CONCLUSIONI

These findings highlight the therapeutic potential of the A40s–miR-340-5p chimera, which exploits hypoxia-associated EphA2 upregulation to selectively target stem-like tumour cells within hypoxic GBM niches. Future studies will assess its in vivo efficacy in orthotopic GBM models.

OP025

CDK4/6 INHIBITORS INDUCE A SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE (SASP) IN ENDOTHELIAL CELLS ENHANCING AGGRESSIVE FEATURES OF TRIPLE NEGATIVE BREAST CANCER (TNBC) CELLS

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INTRODUZIONE

Cellular senescence is a stress-induced and stable cell-cycle arrest leading to both beneficial and detrimental effects. Senescent cells secrete a complex pro-inflammatory secretome, known as the senescence-associated secretory phenotype (SASP), which can promote therapy resistance and tumor progression. Among the senescence-inducing anticancer agents, cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) are highly effective in luminal breast cancer but show a limited efficacy in triple-negative breast cancer (TNBC). Here, we investigated whether CDK4/6i-induced endothelial senescence generates a SASP toward resistance to CDK4/6 inhibition and aggressive features of TNBC.

METODI

Senescence-associated β-galactosidase (SA-β-gal) staining, RNA sequencing, and mass spectrometry followed by dedicated bioinformatics analyses were performed in CDK4/6i-treated human umbilical vein endothelial cells (HUVECs). Moreover, flow cytometry, 3D growth assays, F-actin quantification, polarity index assays, real-time PCR, immunoblotting, and luciferase reporter assays allowed us to evaluate the biological effects and the underlying molecular mechanisms prompted in TNBC cells cultured with conditioned media (CM) from CDK4/6i-treated HUVECs.

RISULTATI

CDK4/6i induced a senescent phenotype in HUVECs, along with the activation of senescence-associated transcriptional programs as well as the acquisition of a SASP profile. Investigating the functional consequences of the endothelial SASP in TNBC cells, we noticed that TNBC cells are responsive to CDK4/6 inhibition. However, CM from senescent HUVECs abolished this TNBC response. Notably, endothelial-derived SASP promoted enhanced proliferative and invasive properties of TNBC cells.

CONCLUSIONI

Our data demonstrate that CDK4/6i induce senescence in endothelial cells, which generates a SASP impairing the sensitivity to CDK4/6 inhibition and stimulating TNBC aggressiveness. Therefore, endothelial SASP may be considered as a novel mechanism involved in TNBC resistance to CDK4/6i and a promising strategy to improve the therapeutic efficacy of CDK4/6i in TNBC patients.

OP026

TAU PROTEIN (MAPT) AS A MARKER OF NEUROBLASTOMA CELL IDENTITY

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INTRODUZIONE

Tau protein, encoded by the MAPT gene, belongs to the family of Microtubule-Associated Proteins (MAPs). Alternative splicing of MAPT generates six isoforms that are differentially expressed during the embryonic development and the postnatal period. Dysregulation of MAPT isoforms expression or post-translational modifications is associated with neurodegenerative diseases. However, Tau biology remains incompletely understood, as several tumors including neuroblastoma (NB) exhibit a differential MAPT expression. NB is a highly heterogeneous extracranial tumor of neuroendocrine origin, and SH-SY5Y cells represent a well-established in vitro model to study cellular differentiation and therapy resistance. In addition, Wnt/ß-catenin signaling contributes to the regulation of NB cell identity. This study aims to investigate whether Tau contributes to the definition of NB cell identity and may serve as a biomarker of distinct cellular states.

METODI

SH-SY5Y cells were used as primary NB cell model. Retinoic acid (RA) and sodium butyrate (NaBu) were employed as differentiation agents in a 6-day protocol. MAPT expression was analyzed by RT-PCR and Western blotting. Immunofluorescence was performed to evaluate protein expression and subcellular localization. Co-immunoprecipitation assays were used to assess the interaction between Tau and ß-catenin.

RISULTATI

MAPT expression was confirmed in NB cell lines IMR-32 and SH-SY5Y predominantly as an embryonic isoform, whereas CHP-126 cells also expressed a postnatal Tau isoform. RA and NaBu treatments induced distinct morphological and molecular changes. In SH-SY5Y cells, Tau and ß-catenin showed increased peri-junctional localization in NaBu-treated cells compared with RA-treated and control cells. This finding suggests a potential Tau-mediated stabilization of ß-catenin, as supported by co-immunoprecipitation assays. These changes are consistent with the emergence of distinct cellular states associated with specific identity features.

CONCLUSIONI

These results support a previously unrecognized role for Tau in modulating NB cell states and differentiation-associated signaling pathways. Tau may contribute to the regulation of NB cell identity, potentially through a crosstalk with Wnt/ß-catenin signaling. MAPT may therefore represent a biomarker for discriminating distinct NB cell identities and associated cell fates, warranting further investigation.

OP027

MODERATE PHYSICAL TRAINING RESCUE DIASTOLIC DYSFUNCTION BY DECREASING MYOCARDIAL FIBROSIS IN A RAT MODEL OF OBESE HFPEF

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INTRODUZIONE

Obesity and obesity related diseases are increasing worldwide due to the spread of western diet and sedentary lifestyle. Obesity has been associated with Heart failure with preserved ejection fraction (HFpEF) which has become more prevalent in recent years. Studying the effects of diet and training on the onset and progression of obesity related HFpEF may be helpful for the development of therapies and lifestyle changes.

METODI

Female Long-Evans rats were divided in sedentary groups with high-fat diet (HFD) and High fat High sugar diet (HFHS) or groups with moderate aerobic training (HFDexe and HFHSexe). A normal chow diet group (CTRL) was used as control. Left ventricle (LV) function and morphology was assessed through echocardiography. The rats were weighted before sacrifice and serum, LV and visceral adipose tissue were harvested. We performed Masson's trichrome staining and immunofluorescence to assess myocardial fibrosis, myocyte cross-sectional area (CSA), coronary artery morphology, capillary density and interstitial senescence. We performed transcriptomic and metabolomic analysis on LV flash frozen tissue samples.

RISULTATI

We show that the diet caused increased bodyweight and VAT accumulation with decreased LV diastolic function and mild hypertrophy. Serum triglycerides and leptin were increased while serum HDL were decreased. Training rescued serum triglycerides, HDL and diastolic function. LV myocardial fibrosis, senescence and arterial wall thickness were found increased in sedentary groups but not in trained ones. LV metabolic profile showed increased glutathione and deoxyadenosine levels while betaine and 2-aminooctanoic acid were decreased. Acetylcarnitine decreased in HFD but was found normal in HFDexe. Transcriptomic analysis shows that HFD and HFHS were associated with increased expression of genes involved in collagen fibers deposition, ETC subunits transcription and decreased genes involved in fatty acid oxidation, calcium voltage gated channels transcription and cytoskeletal motor protein production.

CONCLUSIONI

Our study shows that physical training alone is able to rescue diastolic dysfunction by reducing myocardial fibrosis and coronary vascular remodeling. These changes were associated with metabolomic and transcriptomic profiles associated with increased fatty acid oxidation, better calcium handling, enhanced organelle trafficking, decreased ROS production and decreased collagen related genes activation.

OP028

MEDITERRANEAN DIET-ASSOCIATED EXTRACELLULAR VESICLES ATTENUATE BREAST CANCER AGGRESSIVENESS THROUGH THE MIR-10A-5P/HSP90 AXIS

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INTRODUZIONE

Adherence to the Mediterranean Diet (MD) has been associated with improved outcomes in breast cancer (BC), although the molecular mechanisms remain incompletely understood. Dietary habits may reshape the molecular cargo of extracellular vesicles (EVs), including bioactive molecules such as nucleic acids, proteins and metabolites, potentially influencing pathways involved in BC progression and aggressiveness. Preliminary data showed that EVs from BC patients with high MD adherence attenuate BC cell aggressiveness. Thus, we investigated whether circulating EVs with distinct MD profiles may modulate BC progression through EV-associated miRNAs.

METODI

EVs were isolated and characterized from sera of BC patients stratified according to MD adherence using the MEDAS score into low (L)/medium (M)/high (H) adherence groups. Health-related quality of life was assessed using the EORTC QLQ-C30 questionnaire. RNA sequencing was performed to identify differential miRNA profiles in circulating EVs, which were validated by qRT-PCR in an independent cohort classified according to the same MD adherence criteria. Gain-/loss-of-function approaches were used to investigate the role of selected miRNAs and their target genes in several biological assays.

RISULTATI

miRNA profiling identified distinct EV-associated signatures according to MD adherence, with miR-10a-5p significantly enriched in H-EVs among the different identified miRNAs and validated in an independent cohort. H-EVs increased miR-10a-5p levels in recipient BC cells and reduced the expression of its target gene HSP90. miR-10a-5p mimic and pharmacological HSP90 inhibition counteracted the pro-tumoral effects induced by L-EVs. Increased EV-associated miR-10a-5p levels and reduced spheroid formation were also observed in a patient who shifted from low to high MD adherence during nutritional intervention. High MD adherence was also associated with improved EORTC QLQ-C30 scores.

CONCLUSIONI

Adherence to the MD may reshape the EV-miRNA cargo of BC patients, promoting enrichment of tumor-suppressive miRNAs (i.e. miR-10a-5p). Through modulation of the miR-10a-5p/HSP90 axis, diet-associated EVs may attenuate BC aggressiveness, highlighting a potential molecular link between nutritional interventions and improved BC outcomes.

OP029

HIV-1 PRIMING INDUCES TRAINED IMMUNITY-LIKE FEATURES IN HUMAN MONOCYTES

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INTRODUZIONE

Infants exposed to HIV during gestation but uninfected at birth may display early immune alterations, although the underlying mechanisms remain unclear. To investigate whether HIV-1 exposure can induce trained immunity-like features, we developed an in vitro priming/restimulation model in healthy donor-derived PBMCs and parallel monocyte-enriched cultures.

METODI

Freshly isolated PBMCs from 10 healthy donors were stimulated for 24 hours with or without aldrithiol-2 (AT-2)-inactivated HIV-1. Following stimulus removal, cells were rested for 6 days and subsequently either left untreated or restimulated with LPS for a further 24 hours. The same priming/resting/restimulation protocol was performed in parallel on total PBMCs and on monocytes isolated from PBMCs by plastic adherence. Inflammatory gene expression was assessed by RT-qPCR in isolated monocytes, while intracellular cytokine production was evaluated by flow cytometry in total PBMCs.

RISULTATI

HIV-1 priming followed by LPS re-stimulation induced the strongest transcriptional activation. In particular, IL6 expression was significantly increased in the HIV-1+LPS condition compared with LPS alone ($p<0.05$), IL1B was significantly higher in HIV-1+LPS than in HIV-1 alone ($p<0.05$), and TNFA was markedly upregulated in HIV-1+LPS compared with CTRL ($p<0.001$), LPS ($p<0.05$), and HIV-1 alone ($p<0.05$). Flow cytometric analysis performed on total PBMCs showed a significant increase in CD14+TNFα+ cells in LPS vs CTRL ($p<0.01$), in LPS vs HIV-1 ($p<0.01$), and in HIV-1+LPS vs both CTRL and HIV-1 alone ($p<0.001$).

CONCLUSIONI

These preliminary findings suggest that HIV-1 priming enhances the inflammatory response to secondary LPS challenge, supporting trained immunity-like functional reprogramming in this in vitro model. This system may provide a useful platform to investigate mechanisms through which prenatal HIV-1 exposure contributes to altered early-life immune responsiveness.

OP030

IMMUNOMODULATION OF γδ T CELLS IN GLIOBLASTOMA MULTIFORME

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INTRODUZIONE

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor and remains associated with poor prognosis and limited therapeutic options. Increasing evidence suggests that the tumor immune microenvironment plays a key role in disease progression and resistance to immunotherapy. Among infiltrating immune cells, γδ T lymphocytes represent a promising population because of their cytotoxic and immunomodulatory properties. This study aimed to characterize the phenotype and functional profile of γδ T cells in GBM and their interaction with the tumor microenvironment.

METODI

Tumor tissues and peripheral blood samples from patients with GBM were analyzed by multiparametric flow cytometry to assess γδ T cell subsets, differentiation status and checkpoint receptor expression. Meningioma samples were included as tumor controls. Bulk transcriptomic analyses were performed to evaluate γδ T cell signatures and the expression of butyrophilins and stress-induced ligands. Publicly available single-cell RNA sequencing datasets were analyzed to investigate γδ T cell heterogeneity. Cytokine production was assessed by multiplex Luminex assay using healthy subjects as controls.

RISULTATI

γδ T cells accounted for 1.3–1.8% of tumor-infiltrating lymphocytes and were mainly represented by the Vδ2 subset. Transcriptomic analyses showed overexpression of TRDC together with upregulation of BTN2/BTN3 and stress-induced ligands, including MICA, MICB and ULBP1–6. Tumor-infiltrating Vδ1 cells were enriched in naïve and effector memory subsets, whereas peripheral Vδ2 cells mainly displayed effector memory/TEMRA phenotypes. Single-cell RNA sequencing identified distinct γδ T cell clusters, including one enriched in ADCC-related genes, although this cytotoxic signature appeared reduced in GBM compared with controls. TRGC1/2 expression positively correlated with PDCD1 and HAVCR2, while approximately 35% of γδ T cells co-expressed PD1/TIM3. NKG2C was highly expressed, particularly in tumor-infiltrating Vδ1 cells, whereas NKG2A expression was detected in about 30% of cells. Elevated IL-10 and IL-17A levels supported the presence of an immunomodulatory cytokine milieu.

CONCLUSIONI

Overall, these findings highlight the phenotypic and functional heterogeneity of γδ T cells in GBM and suggest that the immunosuppressive tumor microenvironment may limit their antitumor activity. Understanding γδ T cell modulation in GBM may support the development of immunotherapeutic strategies.

OP031

TCD147 ORCHESTRATES TUMOR GROWTH AND MICROENVIRONMENTAL REMODELING IN H3 K27-ALTERED DIFFUSE MIDLINE GLIOMA

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INTRODUZIONE

Diffuse midline glioma (DMG), H3 K27-altered, is a highly aggressive pediatric brain tumor with a median survival below one year and limited therapeutic options. Beyond epigenetic dysregulation driven by the H3 K27M mutation, tumor–microenvironment interactions contribute to disease progression and therapeutic resistance. CD147 (BSG), a membrane glycoprotein involved in extracellular matrix remodeling and metabolic regulation, is overexpressed in pediatric high-grade gliomas, although its role in DMG remains poorly defined. This study evaluated the therapeutic potential of BSG inhibition in DMG.

METODI

H3 K27-altered DMG cell lines (SF-8628 and SU-DIPG XIII ±P*) were treated with AC-73®, an inhibitor of BSG dimerization. Cell viability, proliferation, clonogenicity, apoptosis, and cell-cycle distribution were assessed by functional assays, western blotting, and flow cytometry. Combination treatment with ONC201 was also evaluated. Antitumor activity was validated in an orthotopic murine model, while tumor microenvironment remodeling was investigated using multiplex Opal assays on the PhenoImager platform.

RISULTATI

AC-73® reduced cell viability with IC50 values of 10–15 μM and significantly impaired proliferation and clonogenicity. Treatment induced G1 arrest through p21/p27 upregulation and pRB reduction, decreased SOX2 and CD133 expression, and activated apoptosis, as demonstrated by increased cleaved caspase-3, caspase-9, and PARP1 levels. AC-73® also showed synergistic activity with ONC201, further reducing DMG cell viability. In vivo, treatment significantly prolonged survival. Spatial profiling revealed microenvironmental remodeling, including reduced CD147# and α-SMA# stromal compartments and altered tumor–stroma interactions. Ly6G#CD68# cells displayed increased spatial distance from CD147#α-SMA# and FAP#α-SMA# stromal populations, while CD147#α-SMA# cells became more separated from CD147#CD68#CD163# macrophages, suggesting disruption of stromal–myeloid coupling and niche reorganization.

CONCLUSIONI

BSG inhibition suppresses DMG growth and promotes tumor microenvironment remodeling, supporting CD147 as a promising therapeutic target for pediatric high-grade gliomas.

OP032

THE ONCOGENIC LNCRNA PVT1 DRIVES DNA DAMAGE RESPONSE ADAPTATION AND THERAPEUTIC VULNERABILITY IN BREAST CANCER

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INTRODUZIONE

Breast cancer (BC) progression relies on adaptive mechanisms enabling tumor cells to tolerate genomic instability and resist therapy. A major determinant of this adaptive fitness is rewiring of DNA Damage Response (DDR), a key hallmark sustaining cancer cell survival under genotoxic stress. In this study, we investigated the molecular mechanisms underlying DDR adaptation, focusing on the role of long noncoding RNAs (lncRNAs), particularly the oncogenic lncRNA PVT1, a critical BC regulator whose high expression is associated with poor clinical outcome in BC patients.

METODI

ERα-positive and ERα-negative BC cell lines were employed. ATAC-Seq, bulk and single-cell RNA-Seq dataset from BC tissues were analysed. Bulk, nascent RNA-seq, and TRRUST analysis were performed following ASO-mediated PVT1 knock-down (kd) in BC cell lines. DDR activation was assessed by monitoring the TUNEL assay, γH2AX accumulation, p53 protein levels, and ATM/ATR activation. ChIRP-Seq analysis revealed specific PVT1-associated genomic loci under basal conditions and following Doxorubicin-induced DNA-damage.

RISULTATI

Multilayered evidence links PVT1 to DDR. TCGA RNA-Seq and ATAC-Seq datasets together with transcriptomic analyses following kdPVT1 in multiple BC cell lines, displayed widespread alterations in p53 and DDR-related signaling pathways. In addition, network analyses further identify key regulators involved in these processes, including p53, PARP1, BRCA1, and ATM. Functionally, PVT1 depletion led to increased DNA damage, as shown by γH2AX accumulation, enhanced TUNEL positivity, and elevated p53, ATM and ATR protein level. Moreover, a ChIRP-seq analysis following doxorubicin treatment revealed dynamic redistribution of PVT1 across the genome, supporting its involvement in adaptive transcriptional responses to genotoxic stress.
To explore the translational relevance of these findings, we applied a perturbation-based approach, displaying that inhibition of PVT1 sensitizes BC cells to DDR-targeting therapies, including ATM and PARP inhibitors promoting synthetic lethality.

CONCLUSIONI

Overall, our work highlights PVT1 as a potential driver of DDR evolution in BC and suggests new opportunities to expand the use of DDR-targeted therapies beyond currently defined genetically selected patient populations.

OP033

A PTX3/TLR4 AXIS SUSTAINS SHH-MEDULLOBLASTOMA GROWTH AND DEFINES A NEW THERAPEUTIC VULNERABILITY

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INTRODUZIONE

Medulloblastoma (MB) is an aggressive neuroectodermal tumor of the cerebellum representing the most common malignant brain tumor in children. MB arises from distinct neural stem cell populations and comprises four distinct subgroups: WNT, SHH, group 3, and group 4. Despite standard treatments (surgery, chemotherapy, and radiotherapy), often associated with severe neurocognitive deficits and long-term neurological damage, 30-40% of MB patients experience recurrence, underscoring the need for novel therapies that balance efficacy with reduced toxicity.
Long Pentraxin 3 (PTX3), an innate immunity component implicated in tumorigenesis, plays different roles in cancer, acting as either an oncosuppressor or a pro-tumoral factor depending on tumor context. Although substantial evidence supports the involvement of PTX3 in various cancers, no data is available about the impact of PTX3 in MB.

METODI

We combined transcriptomic analyses of patient datasets with immunohistochemistry, RNAscope, and protein profiling to evaluate PTX3 expression in MB subgroups. Functional studies were performed in SHH-MB cell lines following PTX3 knockdown or knockout. Effects on proliferation, migration, and angiogenesis were assessed through multiple in vitro assays. In vivo relevance was tested using subcutaneous and orthotopic xenograft models. Mechanistic insights were obtained through phospho-kinase arrays, Western blotting and GSEA. A high-throughput drug screen and combination studies with specific inhibitors and standard chemotherapy (vincristine/cisplatin/cyclophosphamide; VCC) were performed to assess therapeutic potential.

RISULTATI

Our results reveal that PTX3 was strongly enriched in SHH-MB subgroup, where it supported proliferation, motility, and angiogenesis. Mechanistically, PTX3 activated a TLR4-dependent IRAK1/PI3K-Akt/GSK-3/β-catenin signaling cascade. Genetic downregulation or pharmacologic blockade of PTX3-TLR4 significantly reduced tumor growth and angiogenesis in vivo. Moreover, the TLR4 inhibitor TAK-242 markedly impacted SHH-MB growth in vitro, and combination therapy of TAK-242 and VCC produced additive/synergistic effects in vitro and significantly prolonged survival in orthotopic SHH-MB-bearing mice.

CONCLUSIONI

Our findings identify PTX3-TLR4 signaling as key oncogenic driver and a promising therapeutic vulnerability in SHH-MB. Targeting this pathway enhances the efficacy of standard therapy and represents a rationale for mechanism-based combination strategies.

OP034

INVOLVEMENT OF INNATE LYMPHOCYTES IN THE PATHOGENIC MECHANISMS OF COLORECTAL CANCER

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INTRODUZIONE

Colorectal cancer (CRC) is among the most prevalent malignancies worldwide, and a deeper understanding of its immune microenvironment is essential for the development of innovative immunotherapeutic strategies. This study investigated the role of innate lymphoid cells (ILCs), a subset of unconventional lymphocytes involved in tissue homeostasis and immune regulation, in human CRC. Tumor tissues and healthy colorectal mucosa were collected from 30 patients with histologically confirmed adenocarcinoma.

METODI

ILC populations were characterized by multiparametric flow cytometry and confocal microscopy, while the immunomodulatory effects of oleuropein, a natural polyphenol from extra virgin olive oil, were evaluated through functional assays.

RISULTATI

Flow cytometric analyses revealed distinct differences in ILC subset distribution between healthy and tumor tissues. In healthy colorectal mucosa, ILC-3 represented the most abundant subset, whereas tumor samples showed increased frequencies of ILC-1 and a concomitant reduction in ILC-3. This alteration was independent of mismatch repair (MMR) status and appeared to become more pronounced with disease progression, suggesting a potential association between ILC plasticity and tumor development.
Functional analyses demonstrated that ILCs from healthy mucosa responded to inflammatory stimulation by producing IFN-γ, TNF-α, and IL-17A. Oleuropein treatment significantly reduced the production of these cytokines, confirming its anti-inflammatory activity. In contrast, tumor-derived ILCs displayed an impaired functional profile, characterized by the absence of IFN-γ and IL-17A production and the persistence of only low levels of TNF-α. Importantly, oleuropein further reduced TNF-α secretion, indicating that its immunomodulatory effects remain active even within the tumor microenvironment.
Confocal microscopy confirmed the presence of ILC-1, ILC-2, and ILC-3 populations in CRC tissues and revealed potential interactions between CD127# ILCs and CD68# myeloid cells. Co-localization of IL-1R8 and CD56 further suggested the involvement of regulatory inflammatory pathways in shaping local immune responses.

CONCLUSIONI

These findings highlight significant alterations in ILC composition and function in CRC and support the potential value of ILCs as biomarkers and therapeutic targets.

OP035

KCTD14 AS A POTENTIAL MODULATOR OF MITOCHONDRIAL METABOLISM IN THYROID CANCER

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INTRODUZIONE

Members of the potassium channel tetramerization domain (KCTD) protein family have emerged as important regulators in several human cancers. Among them, KCTD14 is poorly characterized and, to the best of our knowledge, its role in thyroid cancer (TC) has not been investigated. This study aimed to investigate the functional role of KCTD14 in TC.

METODI

Public available datasets from The Cancer Genome Atlas Thyroid Cancer were analyzed to assess the expression profile of KCTD14. KCTD14 expression was evaluated by western blot and qRT-PCR in a panel of TC cell lines. KCTD14 levels were modulated in papillary and anaplastic TC cell lines through stable transfection. Migration rate was analyzed by wound healing assay. Label-free proteomics and bioinformatic analyses were performed to identify pathways associated with KCTD14 overexpression in TC. Mitochondrial function was evaluated by Seahorse extracellular flux analysis.

RISULTATI

In silico analyses revealed increased KCTD14 expression in TC tissues compared with normal thyroid counterparts. Functional studies demonstrated that KCTD14 overexpression enhanced cell migration, whereas its silencing exerted opposite effects. Proteomic analysis revealed enrichment of proteins involved in oxidative phosphorylation and mitochondrial metabolism in KCTD14-overexpressing cells. Consistently, Seahorse analysis showed increased mitochondrial respiratory capacity and enhanced oxidative metabolism upon KCTD14 overexpression.

CONCLUSIONI

Our preliminary findings suggest that KCTD14 may contribute to TC progression by modulating mitochondrial metabolism.

OP036

P2RX4 DRIVES LIPID METABOLIC REWIRING AND PSEUDOHYPOXIC ADAPTATION IN CCRCC

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INTRODUZIONE

Clear cell renal cell carcinoma (ccRCC) is driven by loss of Von Hippel–Lindau causing persistent pseudohypoxic state and metabolic rewiring resulting in lipid biosynthesis, phospholipid remodeling and mitochondrial adaptation, linked to poor prognosis and therapy resistance. In patient datasets, purinergic signaling correlates with hypoxia and lipid metabolism and P2RX4 expression aligns with both. We investigated P2RX4-driven metabolic rewiring in ccRCC and its therapeutic relevance combined with HIF-2α inhibition.

METODI

ccRCC cell lines were cultured in 2D and 3D systems and treated with P2RX4 inhibitor alone and in combination with HIF-2α blockade. Stable P2RX4 overexpression was generated in normal renal epithelial cells. Integrated transcriptomic, metabolomic and lipidomic analyses were performed in P2RX4-overexpressing cells. Patient datasets were additionally interrogated to assess metabolite and pathway signatures in clinical samples. Key metabolic regulators, SLC25A1, ATP citrate lyase, Acetyl-CoA carboxylase, CPT2 and GDE1 were assessed. Functional validation was performed in xenograft and PDX models.

RISULTATI

Overexpression of P2RX4 induced pseudohypoxia and lipid remodeling. Integrated analyses showed increased glycolytic flux, lactate accumulation and enhanced TCA cycle activity. Carbon was progressively redirected toward biosynthetic pathways through increased citrate export via SLC25A1, linking mitochondrial metabolism to cytosolic acetyl-CoA availability. This supported a lipogenic program driven by ACLY/Acetyl-CoA carboxylase axis, promoting malonyl-CoA production and sustained lipid synthesis. In parallel, CPT2 and GDE1 indicated coordinated regulation of fatty acid oxidation and phospholipid turnover. Inhibition of P2RX4 reversed this metabolic state, reducing TCA-to-lipogenesis carbon flux, suppressing ACC activation and limited lipid synthesis. These effects were validated in tumor cells and patient-derived tissues. Notably, P2RX4-associated metabolic signatures correlated with patient phenotype. Combined inhibition of P2RX4 and HIF-2α significantly reduced tumor growth and attenuated hypoxia-driven metabolic programs in 3D spheroids, xenografts and PDX models.

CONCLUSIONI

P2RX4-mediated Ca^{2+} signaling amplifies pseudo-hypoxic adaptation in ccRCC, sustaining lipid metabolic reprogramming. Combined targeting of P2RX4 and HIF-2α enhances anti-tumor efficacy, supporting purinergic calcium signaling as a combinatorial therapy in VHL-deficient renal cancer.

OP037

SP6 PROMOTES LPCAT1-DEPENDENT LYSOLECITHIN REPROGRAMMING AND PRESERVES CARDIOMETABOLIC HOMEOSTASIS IN TYPE 2 DIABETES

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INTRODUZIONE

Alterations in lysophosphatidylcholine (LPC) and phosphatidylcholine (PC) metabolism have been implicated in endothelial dysfunction, insulin resistance, and impaired tissue repair. However, the contribution of lysolecithin remodeling to the development of type 2 diabetes mellitus (T2DM) and its systemic consequences remains poorly understood. This study investigated tissue-specific LPC/PC remodeling in T2DM and evaluated the effects of SP6, an orally bioavailable peptide derived from Spirulina platensis.

METODI

T2DM was induced in mice through high-fat diet and streptozotocin administration. SP6 was orally administered throughout the study. Lipidomic profiling of plasma and tissues was performed by UHPLC-HRMS. Endothelial responses were evaluated through wound-healing assays, membrane fluidity analysis, immunofluorescence, immunoblotting, and gene expression analyses. Tissue expression of LPCAT1 and glucose transporters was assessed in pancreas, liver, skeletal muscle, and adipose tissue. Pancreatic insulin distribution was further investigated by MALDI mass spectrometry imaging.

RISULTATI

High-glucose exposure induced a marked disruption of LPC/PC homeostasis, associated with increased membrane fluidity, reduced endothelial repair capacity, altered VE-cadherin localization, and downregulation of LPCAT1. These changes were accompanied by impaired VEGF/API5 signaling and dysregulated GLUT1 trafficking. In diabetic mice, SP6 preserved LPCAT1 expression across multiple metabolic tissues and promoted a selective remodeling of circulating LPC species, particularly LPC 16:0, LPC 18:0, and LPC 20:4. SP6 treatment was associated with improved glycemic control, preservation of pancreatic insulin content, and normalization of metabolic and endothelial molecular signatures.

CONCLUSIONI

These findings reveal tissue-specific lysolecithin reprogramming during T2DM development and identify LPCAT1-mediated LPC/PC homeostasis as a key mechanism underlying disease progression and SP6-mediated protection. By modulating lipid metabolism, vascular integrity, and glucose regulation, SP6 restores endothelial and metabolic function, highlighting lysolecithin remodeling as a novel therapeutic target and supporting SP6 as a promising preventive strategy for T2DM and its cardiometabolic complications.

OP038

ROLE OF VERSICAN PROTEOGLYCAN IN BREAST CANCER ADIPOSE MICROENVIRONMENT

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INTRODUZIONE

Mammary adipose tissue (MAT) is a key component of the tumor microenvironment, contributing to breast cancer (BC) progression. MAT-mesenchymal/stromal stem cells (MAT-MSCs) can be reprogrammed by BC cells; however, the mechanisms underlying double bi-directional interaction still remain poorly understood. We recently showed that Versican proteoglycan (Vcan) is upregulated in MAT and correlates with tumor Ki67 expression in luminal BC specimens. This study aimed to assess whether Vcan production by MAT-MSCs may play a role in MAT-MSC–BC cross-talk.

METODI

MAT-MSCs were isolated from MAT biopsies of healthy women (n=18), and Vcan expression was assessed by RT-PCR and ELISA. For co-culture experiments, three MAT-MSC isolates with high (MSCs-Vcan+) and three with low (MSCs-Vcan-) Vcan expression were selected and co-cultured with MCF7 breast cancer cells (ER+). 2D co-cultures were established using transwell system and mRNA levels were analyzed by RT-PCR. 3D co-cultures were generated in ultra-low attachment plates, and spheroid size was measured using Fiji-ImageJ at 3, 6, 10 days. In MSCs-Vcan+–MCF7 3D co-cultures, Vcan expression was silenced using two independent siRNAs, with scrambled siRNA as control. Spheroid surface analysis was performed at day 3.

RISULTATI

RT-PCR and ELISA analyses identified MAT-MSCs as a source of Vcan and enabled their stratification into high- and low-proteoglycan-expressing cells. In 2D co-cultures, high Vcan levels promoted cross-talk with MCF7 cells, increasing pro-angiogenic (VEGF, $p < 0.05$) and inflammatory/fibrotic markers (IL6, αSMA, FAP; all $p < 0.05$) in MAT-MSCs, and MMP9 and MMP2 in MCF7 cells (both $p < 0.005$). In 3D co-culture experiments, MSCs-Vcan+ formed tumor spheroids with a larger surface compared to MSCs-Vcan- (day 3, day 6, and day 10; all $p < 0.0001$). Finally, silencing Vcan gene expression in MSCs-Vcan+–MCF7 3D co-cultures resulted in a consistent reduction in spheroid surface area at day 3 compared to MSCs-Vcan+–MCF7 spheroids treated with scrambled siRNA.

CONCLUSIONI

MAT-MSCs locally interact with BC cells, promoting a tumor-supportive microenvironment. Vcan production appears to play a key role in this cross-talk, enhancing BC cell aggressiveness.

OP039

KCASH2 AS A NEW MODULATOR OF THE MITOTIC CHECKPOINT

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INTRODUZIONE

MAD2 is the main player in the spindle assembly checkpoint (SAC), essential for chromosomal stability during cell mitosis and preventing defected cellular divisions that may lead to aneuploidy. Altered MAD2 protein levels have been reported in several human tumors characterized by chromosomal instability. Recently, MAD2 was identified as a new interactor of KCASH2, a tumor suppressor of the Hedgehog pathway identified in our laboratory. Characterizing the KCASH2–MAD2 interaction could provide new insights into the regulation of MAD2 activity and mitotic checkpoint control.

METODI

Co-immunoprecipitation and Western blot analyses were performed to assess KCASH2–MAD2 interaction and MAD2 protein levels. To further characterize the KCASH2–MAD2 interaction, AlphaFold Multimer was used in a fragmentation-based in silico strategy to predict the binding of KCASH2-derived regions to MAD2. The predicted interface, refined by molecular dynamics data, guided the design of minimal KCASH2-derived peptides, which were synthesized as resin-bound probes for in vitro MAD2-binding assays and as FITC-conjugated peptides to monitor their intracellular distribution. The effects of KCASH2 on cell-cycle progression and mitotic defects were assessed by FACS analysis and immunofluorescence microscopy.

RISULTATI

KCASH2 was found to bind MAD2 and promote its proteasome-dependent degradation, thereby reducing MAD2 protein levels. A minimal peptide derived from the C-terminal region of KCASH2 retained the ability to bind MAD2, supporting the relevance of this region in the KCASH2–MAD2 interaction. Notably, both KCASH2 overexpression and silencing impaired cell-cycle progression, leading to a prolonged metaphase-to-anaphase transition and increased mitotic defects, including lagging chromosomes, micronuclei and anaphase bridges.

CONCLUSIONI

This study identifies KCASH2 as a new regulator of the mitotic checkpoint. Characterization of the KCASH2–MAD2 interaction and identification of a minimal functional peptide provide new insights into SAC regulation and may support future therapeutic strategies for tumors characterized by chromosomal instability.

OP040

ASPARAGINASE INDUCES AMINO ACIDS-DEPENDENT METABOLIC STRESS COUPLED WITH ABORTIVE S-PHASE AND LOSS OF CELL-MATRIX INTERACTIONS IN RENAL CELL CARCINOMA

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INTRODUZIONE

Renal cell carcinoma is a highly metabolic-dependent tumor characterized by profound reprogramming of biosynthetic and redox fluxes and therefore represents an ideal model for mechanistically study of the action of anti-metabolism drugs. Asparaginase, a landmark drug for acute lymphoblastic leukemia, systemically disrupts amino acid homeostasis, with effects that go beyond simple asparagine removal.
We have previously shown that, in 786-O renal cell carcinoma, asparaginase induces an S-phase abortion phenotype, characterized by activation of replicative stress markers without quiescent arrest. Here we demonstrate that this defect is not supported by nucleotide deficiency: exogenous nucleotides addition does not restore DNA synthesis, while glutamine results in partial recovery. These results indicate that asparaginase induces upstream metabolic collapse rather than simple nucleotides depletion.

METODI

Through single-cell RNA sequencing analyses, we identify a heterogeneous transcriptomic response dominated by a strong activation of oxidative and metabolic stress programs (TXNIP, HMOX1), a concomitant suppression of mitochondrial antioxidant defenses (SOD2, CAT, UCP2), an incomplete activation of the Integrated Stress Response and a robust increase in stress replication signals (ATR, CDKN1A). Moreover, a clear downregulation of adhesion-related genes (CD44, DPP4, STAT1) is evident and real-time adhesion assays revealed a significant reduction in cell adhesion following asparaginase treatment.

RISULTATI

We propose a model in which asparaginase imposes a glutamine-dependent metabolic stress condition that decouples biosynthetic demand from cellular metabolic capacity, preventing the maintenance of functional and balanced survival. Cells enter S-phase without completing DNA replication, undergoing replicative collapse rather than quiescent adaptation, while concurrently displaying impaired adhesion capacity due to downregulation of CD44. The impairment of cell adhesion supports the existence of a coordinated program coupling metabolic stress with loss of cell–matrix interaction, resulting in overall loss of cell fitness.

CONCLUSIONI

These findings redefine the mechanism of action of asparaginase in renal cell carcinoma, highlighting a specific metabolic vulnerability related to glutamine dependence.

OP041

CELL STATE PLASTICITY HIGHLIGHTS THE STING PATHWAY AS A THERAPEUTIC VULNERABILITY FOR HIGH-RISK NEUROBLASTOMA

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INTRODUZIONE

Neuroblastoma (NB), is a pediatric extracranial solid malignancy and frequently “cold” tumor, characterized by low chemokine expression and lack of immune infiltration. Epigenetic plasticity between adrenergic (ADRN) and mesenchymal-like (MES) states shapes therapeutic responsiveness in high-risk neuroblastoma, with the MES state conferring chemoresistance while displaying enhanced immunogenic and proinflammatory features. cGAS-STING pathway is a conserved innate immune signaling axis frequently impaired in cancer, whose restoration may be crucial to convert “cold” into “hot” tumors.

METODI

In-depth scRNA-seq data analysis, RT-PCR and WB were used to evaluate STING pathway expression and functionality. In silico analysis and pyrosequencing allowed evaluation of cGAS/STING promoter methylation. Immune responses were assessed via NK cell degranulation and killing assays.

RISULTATI

scRNA-sequencing of human NBs indicates that cGAS and STING are almost undetectable in ADRN neuroblastic cells, as compared to other cells in the tumor microenvironment, while the TBK1 and IRF3 downstream signaling elements are broadly expressed across cell types. In NB preclinical models, STING pathway activity is cell-state dependent: ADRN-state cells repress cGAS and STING expression, whereas STING, and occasionally cGAS, are expressed in MES-state cells. Mechanistically, MES-to-ADRN transition represses STING through DNMT1-dependent promoter methylation, while ADRN-to-MES transition restores its expression. Although ADRN cells remain refractory to STING agonists even upon enforced STING expression, indicating stringent pathway suppression, the MES state supports functional STING activation, inducing type I interferon and proinflammatory programs and promoting antitumor natural killer (NK) cell activation. Accordingly, supernatants from STING-activated NB lines and MES-enriched cells obtained from temozolomide/irinotecan-treated patient-derived xenograft trigger NK cell degranulation and tumor cell killing.

CONCLUSIONI

These findings identify NB cell-state plasticity as a determinant of STING pathway competence and support exploiting STING signaling to target the chemoresistant MES state through sequential or combinatorial immunomodulatory strategies.

OP042

A ROLE OF THE UBIQUITIN LIGASE PRAJA2 IN AUTOPHAGY AND METABOLIC REMODELLING OF GBM CELLS.

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INTRODUZIONE

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adult population. Its high recurrence rate and resistance to current therapies underscore the urgent need for novel treatment strategies.
Autophagy, a highly conserved process activated under nutrient stress, is frequently dysregulated in aggressive human cancers, including GBM, where it regulates tumor growth and metabolic adaptation to stress conditions. The ubiquitin system has a key regulatory role in the autophagic pathway, thereby influencing cancer progression. In this context, a relevant role is played by the E3 ligase praja2 that is markedly upregulated in GBM and its expression is mechanistically linked to the growth and progression of GBM. The aim of this study is to demonstrate that praja2, by regulating the autophagic pathway, controls the growth and metabolism of GBM.

METODI

To this end, we used an integrated approach of proteomic and transcriptomic analyses, to investigate whether praja2 interacts with and regulates components and regulators of autophagic and metabolic pathways. Furthermore, we used gene-targeting strategies, biochemical assays, in situ immunostaining and metabolic profiling to demonstrate that praja2 effectively binds to- and ubiquitylates components of autophagy machinery, thus affecting GBM growth and metabolism.

RISULTATI

We identified a praja2-centered molecular network operating in GBM cells that involves components and regulators of aerobic glycolysis (Warburg effect), RNA metabolism and catabolic pathways. We show that Praja2, thus, interacts with- and ubiquitylates core components of the autophagy machinery, markedly impacting on GBM growth and cancer metabolic reprogramming.

CONCLUSIONI

In conclusion, our findings advance the understanding of the praja2 function in GBM growth, providing insights in the role of this ligase in the autophagic pathway and metabolic adaptation of GBM cells.

OP043

MDM2 INHIBITION ENHANCES NK CELL-MEDIATED CYTOTOXICITY AGAINST OSTEOSARCOMA THROUGH P53-DEPENDENT UPREGULATION OF ACTIVATING RECEPTOR LIGANDS

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INTRODUZIONE

Osteosarcoma (OS) remains a challenging malignancy with limited treatment options. Natural Killer (NK) cells represent a promising immunotherapeutic approach, yet their efficacy against tumor cells can be limited by poor recognition of target ligands. MDM2 inhibition restores p53 function, which acts as a transcription factor for genes encoding NK cell-activating receptor ligands, potentially enhancing NK cell-mediated cytotoxicity.

METODI

Three OS cell lines with distinct p53 status (SJSA-1: MDM2-amplified/p53 wild-type; U2OS: p53/MDM2 wild-type; SAOS-2: p53-null) were treated with MDM2 antagonists (RG7112, Idasanutlin, Siremadlin) for 24-48 hours. p53 pathway reactivation was assessed by Western blotting. Flow cytometry was used to quantify expression of NK cell-activating receptor ligands (NKG2D, DNAM-1), death receptors (Fas, TRAIL-R2), and adhesion molecules. NK cell degranulation was measured by CD107a staining following co-culture with drug-treated OS cells. Three-dimensional spheroid models were employed to assess NK cell infiltration and apoptosis induction in more physiologically relevant conditions.

RISULTATI

MDM2 inhibition induced dose-dependent increases in p53 and its downstream targets MDM2 and p21 in p53 wild-type cell lines but not in p53-null SAOS-2 cells. Treatment resulted in significant upregulation of NKG2D ligands (MICA/B, ULBP1, ULBP2) and DNAM-1 ligand (PVR) across OS cell lines. NK cells co-cultured with MDM2td-pretreated OS cells displayed enhanced degranulation, with the most pronounced effect observed in the MDM2-amplified SJSA-1 line. Combined treatment with MDM2 inhibitors and anti-PD-1 checkpoint blockade (Pembrolizumab) significantly potentiated NK cell cytotoxic activity. In 3D spheroid models, MDM2 inhibition enhanced NK cell infiltration and apoptosis induction, with combined treatment resulting in significant spheroid size reduction.

CONCLUSIONI

These findings demonstrate that pharmacological MDM2 antagonism enhances NK cell recognition and cytotoxic activity against OS cells by restoring p53-dependent expression of NK cell-activating ligands. The synergistic effect with immune checkpoint inhibition suggests a rational combinatorial strategy for NK cell-based immunotherapy in osteosarcoma. These preclinical results support clinical translation of MDM2 inhibitors combined with adoptive NK cell transfer for solid tumors.

OP044

THE ANTIDIABETIC DRUG METFORMIN INHIBITS COLORECTAL CANCER GROWTH THROUGH THE PEN2–LYSOSOME–NRF2 AXIS

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INTRODUZIONE

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide and is characterized by high incidence, recurrence, and resistance to therapy. In addition to genetic and epigenetic alterations, CRC progression critically depends on metabolic rewiring, which represents an exploitable therapeutic vulnerability. Here, we investigated the antitumor activity of metformin, a widely used antidiabetic drug, focusing on a recently described lysosomal mechanism involving PEN2.

METODI

Metformin antitumor activity was evaluated in syngeneic allograft and APCdel intestinal polyposis mouse models treated by oral gavage. Human and murine CRC cell lines, murine 3D cultures, and patient-derived tumor organoids were exposed to metformin to assess proliferative and therapeutic responses. PEN2 loss-of-function models were generated by shRNA knockdown and CRISPR–Cas9 editing. Cellular metabolism was analyzed through Seahorse and Omnilog bioenergetic assays, while mitochondrial dynamics and lysosomal function were assessed by confocal and electron microscopy.

RISULTATI

Using in vitro and in vivo CRC models, we show that metformin exerts a strong antitumor effect at clinically relevant low micromolar doses. At these concentrations, metformin does not inhibit mitochondrial Complex I, but instead disrupts lysosomal acidification. Since lysosomal dysfunction affects mitochondrial homeostasis and redox balance, we investigated the downstream effects of PEN2-dependent lysosomal impairment, observing mitochondrial stress, increased ROS production, and marked antiproliferative activity. Consistent with the role of lysosomes in autophagy, PEN2 depletion impaired autophagic flux, identifying PEN2 as a key mediator of tumor-suppressive lysosomal signaling in CRC. Metformin also increased NRF2 levels, whereas this effect was abolished by PEN2 loss, supporting NRF2 as a downstream effector of the PEN2-dependent lysosomal stress response.

CONCLUSIONI

Overall, these findings define a PEN2–lysosome–mitochondria–ROS–NRF2 signaling axis as a mediator of metformin's anticancer activity. This work provides mechanistic insight into metformin's action at clinically achievable doses and supports lysosomal signaling pathways and PEN2 as promising therapeutic targets in CRC.

OP045

MODULATION OF TH1 AND TH2 CHEMOKINES SECRETION BY CORTICOSTEROIDS, OR RAPAMYCIN, IN PRIMARY ORBITAL CELL CULTURES OF PATIENTS WITH THYROID EYE DISEASE

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INTRODUZIONE

Thyroid eye disease (TED) is a disfiguring and vision-threatening autoimmune disease of the retroocular tissues occurring in patients with Graves' disease. In the immunopathogenesis of TED, autoantibodies against TSH receptor (TSH-R) and IGF receptor-1 (IGF-1R) are generated by the autoimmune process. TSH-R and IGF-1R autoantibodies target orbital fibroblasts (OF), key mediators of orbital inflammation.
Cytokines recruit inflammatory cells into orbital tissues and activate OF, which proliferate, and differentiate into adipocytes and myofibroblasts. The initial active phases of orbital infiltration are led by T helper 1 (Th1) immune predominance, that switches toward a Th2 immune response in the late/inactive phase of the disease. Indeed, activated Th1 lymphocytes induce IFN-γ and TNF-α synthesis, which in turn stimulates orbital cells to release Th1 chemokines, reiterating the autoimmune process.
This study aimed to evaluate the effects of corticosteroids or rapamycin on the secretion of chemokines in TED orbital cells.

METODI

We evaluated the effect of increasing concentrations of corticosteroids or rapamycin on the secretion of the prototype Th1 CXCL10 (C-X-C motif chemokine 10) and Th2 CCL2 (C-C Motif Chemokine Ligand 2) chemokines, in primary cultures of fibroblasts, preadipocytes and myoblasts from TED patients undergoing orbital surgery.

RISULTATI

Regarding CXCL10 secretion: 1) CXCL10 was not detectable in the supernatant; 2) IFN-γ induced a dose-dependent CXCL10 release, whereas TNF-α alone had no effect; 3) TNF-α plus IFN-γ had a significant synergistic effect on CXCL10 secretion.
Concerning CCL2 secretion: 1) in basal condition CCL2 was secreted in low amounts; 2) TNF-α dose-dependently induced CCL2 release, while IFN-γ alone had no effect; 4) TNF-α plus IFN-γ had a significant synergistic effect on CCL2 secretion.
Moreover, the addition of corticosteroids, or rapamycin (in a pharmacological range), upon stimulation with IFN-γ and TNF-α, dose-dependently reduced the release of CXCL10 and CCL2 in TED orbital cells.

CONCLUSIONI

Corticosteroids, or rapamycin, have an inhibitory effect on the secretion of both Th1 and Th2 chemokines, in TED orbital cells, suggesting that their therapeutic role could be obtained, at least in part, through the modulation of chemokines secretion.

OP046

MCUB LIMITS INTESTINAL EPITHELIAL REGENERATION AND BARRIER INTEGRITY DURING INFLAMMATORY BOWEL DISEASE

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INTRODUZIONE

Inflammatory bowel disease (IBD) is characterized by chronic intestinal inflammation, disruption of the epithelial barrier, and defective mucosal healing. Mitochondrial calcium uptake, mediated by the mitochondrial calcium uniporter (MCU) complex, couples cytosolic calcium signals to mitochondrial bioenergetics, thereby regulating ATP production, oxidative metabolism, and adaptive stress responses. MCUb is an inhibitory paralog of the pore-forming MCU subunit that reduces the calcium permeability of the uniporter complex and limits mitochondrial calcium uptake. Given the pivotal role of mitochondrial function in epithelial homeostasis, we investigated the regulation and functional significance of mitochondrial calcium in intestinal epithelial cells during colitis.

METODI

We used intestinal organoids derived from iPSCs and treated with conditioned medium from M1-polarized macrophages. Experimental colitis was induced in mice by dextran sulfate sodium (DSS) administration.

RISULTATI

We found that MCUb expression is robustly induced in intestinal epithelial cells in response to inflammatory stimuli, both in experimental colitis and following cytokine exposure. To determine the physiological significance of this response, we examined the susceptibility of MCUb-deficient mice to experimental colitis. Genetic deletion of MCUb markedly enhanced epithelial regeneration following intestinal injury, leading to accelerated restoration of the colonic mucosa. Consistently, MCUb-deficient mice exhibited improved epithelial integrity, reduced tissue damage, and enhanced barrier preservation compared with wild-type littermates.

CONCLUSIONI

These findings identify MCUb as a key regulator of the epithelial response to intestinal inflammation. By limiting mitochondrial calcium uptake, MCUb restrains epithelial regeneration and delays mucosal repair following colitic injury. Collectively, our results support a model in which inflammation-induced MCUb expression contributes to defective epithelial healing and highlight MCUb as a potential target for promoting mucosal regeneration in IBD.

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