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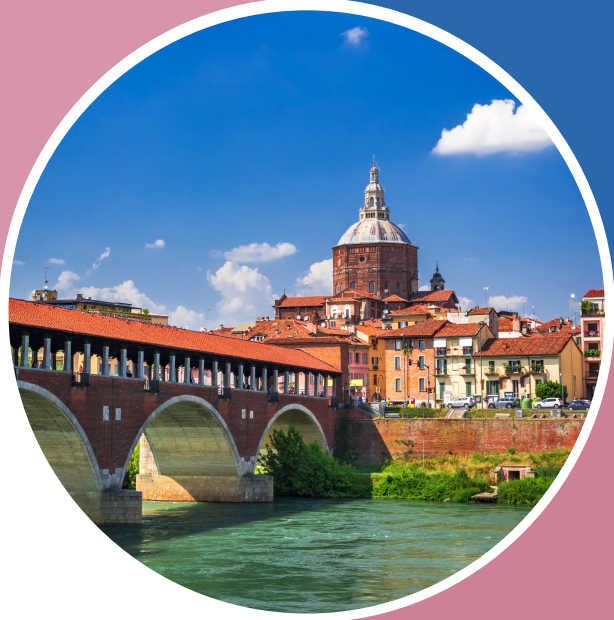

ABSTRACT BOOK

P001

TARGETING EIF4A1 IS EFFECTIVE AGAINST HUMAN INTRAHEPATIC CHOLANGIOCARCINOMA

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BACKGROUND-AIM

Intrahepatic cholangiocarcinoma (iCCA) is the second most frequent primary liver tumor, characterized by clinical aggressiveness, dismal outcome, and limited therapeutic options. Thus, innovative treatments are urgently required to improve the prognosis of iCCA patients significantly.

METHODS

In this study, we have determined the pathogenetic and therapeutic role of eukaryotic initiation factor 4A1 (EIF4A1), a subunit of the eIF4F complex involved in translation initiation, in human iCCA.

RESULTS

We found that preinvasive (n=12), invasive (n=162), and metastatic (n=14) iCCA lesions exhibit ubiquitous eIF4A1 upregulation. Additionally, eIF4A1 mRNA levels from 42 specimens showed a significantly higher expression in iCCA samples compared to non-tumorous tissues ($p<0.0001$) and in large duct type lesions ($p=0.020$). Also, eIF4A1 expression was inversely associated with patients' prognosis ($p<0.001$). Moreover, Zotatifin, an eIF4A1 specific inhibitor in clinical trials, significantly reduced the growth of iCCA cell lines, iCCA cancer-associated fibroblasts (CAFs), and patient-derived tumor organoids. At the metabolic level, Zotatifin decreased glycolysis of iCCA cells without affecting mitochondrial respiration. Furthermore, the Bcl-xl inhibitors A-1155463 and DT2216 profoundly augmented apoptotic cell death when administered in association with Zotatifin.

CONCLUSIONS

The data highlight eIF4A1 as a potential target for treating human iCCA. Combined inhibition of eIF4A1 and Bcl-xl may offer an effective therapeutic strategy against this deadly disease.

P002

IMMUNE LANDSCAPES OF COLORECTAL CANCER: UNVEILING PROGNOSTIC SIGNATURES THROUGH TRANSCRIPTOMIC DECONVOLUTION

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BACKGROUND-AIM

Colorectal cancer (CRC) is a leading cause of cancer-related morbidity and mortality worldwide. Despite therapeutic advances, its pronounced molecular heterogeneity and the dynamic nature of the tumor microenvironment (TME) continue to hamper accurate prognosis and individualized treatment strategie

METHODS

In this study, we performed RNA-Seq analysis on 131 matched pairs of primary CRC tumors and adjacent normal colonic tissues (NCT), followed by computational deconvolution to estimate immune cell composition within the TME.

RESULTS

We identified significant alterations in immune cell subpopulations between tumor and normal tissue. CRC samples showed enrichment in M0 and M1 macrophages, activated mast cells, activated dendritic cells, and CD4+ memory T cells. In contrast, normal tissues harbored higher levels of CD8+ T cells, resting mast cells, memory B cells, plasma cells, and M2 macrophages. Crucially, several of these populations were significantly associated with overall survival (OS). Higher proportions of plasma cells, naïve B cells, and resting CD4+ memory T cells correlated with improved OS, while elevated levels of monocytes, M2 macrophages, activated mast cells, and neutrophils were linked to poorer outcomes. Notably, activated dendritic cells emerged as strong predictors of favorable prognosis, suggesting a central role in mounting effective anti-tumor immunity.

CONCLUSIONS

These findings indicate that specific immune signatures, as inferred by deconvolution, may serve as independent prognostic biomarkers and guide immunotherapeutic strategies. Correlations between immune composition and gene expression–based risk scores highlight the potential of integrating immunological and molecular data for more precise patient stratification. Our study underscores the critical value of immune profiling in refining CRC prognosis and advancing personalized treatment approaches.

P003

ESTABLISHING A HIGH-QUALITY LABORATORY WITH HELP FROM CAP

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BACKGROUND-AIM

The College of American Pathologists (CAP) has been active in the improvement of laboratory quality for nearly 80 years. The collective expertise of its more than 18.000 members - every one of them a board-certified anatomic or clinical pathologist - is available to all laboratories worldwide. We will show how to find and make best use of this knowledge and demonstrate pathways to improve the quality of laboratory results and the efficiency of the laboratory itself.

METHODS

The collective expertise of the CAP's membership is laid down in the norms along which we accredit laboratories all over the globe, called the checklists. These checklists are discipline-specific, highly detailed, updated every year and continue to be at the forefront of laboratory quality science.

RESULTS

Outside the US, CAP accreditation is a voluntary process driven by the need to improve laboratory result quality and patient safety. We can show improvements in participating laboratories relating to
- reduction of false results
- reduction of repeat testing
- increased laboratory personnel qualification and education
- increased laboratory personnel motivation and pride

CONCLUSIONS

Laboratory quality is a strategically important topic and investment. The benefits of it can be seen on economic and patient health scales. The CAP is a helpful guide to higher laboratory quality.

P004

INTERLEUKIN-1 RECEPTOR 8 DEFICIENCY ENHANCES THE ANTI-TUMOUR ACTIVITY OF DOXORUBUCIN AND REDUCES CARDIOTOXICITY

F. Scavello [1], D. Supino [1], A. Felicetta [1], F. Vaino [1], S. Carnevale [1], G. Pezzone [1], I. Di Ceglie [1], A. Mariancini [1], A. Rigatelli [1], F. Pasqualini [1], A. Idini [1], A. Priviterra [1], M. Rubino [1], E. Magrini [1], S. Jaillon [1], C. Greco [1], G. Condorelli [1], A. Mantovani [1], C. Garlanda [1]

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BACKGROUND-AIM

Chemo-immunotherapy, combining anthracyclines like Doxorubicin (Doxo) with immune checkpoint inhibitors (ICI), enhances immunogenic cell death and tumor-specific immune responses against different types of cancer. It increases the risk of severe side effects, such as inflammation, hepatotoxicity, and T cell-mediated cardiotoxicity. Interleukin-1 (IL-1) plays a key role in both cancer- and cardiac-related inflammation. IL-1 receptor 8 (IL-1R8), a negative regulator of IL-1 and TLR signaling, functions as an IC for NK and CD8+ cells, enhancing anti-tumor immunity in murine models. To explore IL-1R8 as a novel IC, we investigated the role and potential side effects of its deficiency in combination with chemotherapy.

METHODS

We investigated the impact of IL-1R8 deficiency on tumor growth and cardiac toxicity in response to Doxo treatment. Wild-type (wt) and Il1r8-/- mice were subcutaneously transplanted with FS6 fibrosarcoma cells and treated with high or low (20-10 mg/kg) Doxo concentration. Tumor growth was monitored and immune infiltration was analyzed. Cardiac function and fibrosis, immune infiltration, and inflammation were examined in cardiac tissue.

RESULTS

The high-dose regimen completely eradicated the tumor in both genotypes, while low-dose Doxo significantly reduced tumor size in Il1r8-/- mice, accompanied by more mature CD8+ T cells, compared to wt mice. High-dose Doxo impaired cardiac function, with no genotype differences, indicating that IL-1R8 blockade does not impact on cardiotoxicity. In contrast with the hypothesis, Il1r8-/- mice exhibited reduced cardiac fibrosis, lower inflammation, and fewer CD4+ cells in cardiac tissue after Doxo treatment, compared to wt counterpart. Co-culture experiments (CD4+Th1 cells plus cardiac fibroblasts or macrophages) revealed that Il1r8-/- Th1 cells had reduced pro-fibrotic and inflammatory activity compared to wt cells, in line with previous study reporting MyD88 is a regulator of cardiac fibrosis through modulation of T-Cell activation.

CONCLUSIONS

Our results show that IL-1R8 targeting enhances the anti-tumor activity of Doxo, while reducing cardiotoxicity and fibrotic effects of CD4+ T cells.

P005

TARGETING GLUTAMINE SYNTHETASE OVERCOMES THE RESISTANCE OF PROSTATE CARCINOMA CELLS TO GLUTAMINE DEPLETION

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BACKGROUND-AIM

Prostate carcinoma (PCa) cells are strongly dependent on glutamine (Gln) in vitro, but clinical trials targeting Gln metabolism have been disappointing. Since this discrepancy could be attributable to the unrealistic culture conditions adopted, here we have studied Gln dependence of PCa cells under in vitro conditions mimicking the in vivo environment.

METHODS

Androgen-independent (PC-3 and DU145) and androgen-dependent (LNCaP and VCaP) PCa cell lines were used. Cells were cultured in the conventional medium DMEM, which contains supraphysiological Gln levels and lacks Asn, Glu and Pro, or in Plasmax™, an advanced medium that includes all the amino acids at physiological concentration. LC-MS was used for determining amino acid levels.

RESULTS

PCa cells exhibited net Gln influx in both media, but upon Gln deprivation the loss of viability was much larger in DMEM than in Plasmax, with the two androgen-independent cell lines more Gln-dependent than LNCaP and VCaP cells. Upon Gln starvation, the decline in cell Gln up-regulated Glutamine Synthetase (GS) in all PCa cells regardless of the medium adopted. However, only in Plasmax GS silencing or its inhibition by L-methionine sulfoximine (MSO) further reduced cell Gln levels and hindered proliferation. Notably, in Gln-depleted Plasmax, PCa cells increased the consumption of Asn. Consistently, deprivation of both Gln and Asn had much larger suppressive effects on cell growth than deprivation of the sole Gln. Consistently, the combined treatment with L-Asparaginase, which hydrolyzes extracellular Gln and Asn, and the GS inhibitor MSO caused a marked drop in the intracellular levels of both amino acids, a significant inhibition of proliferation along with cell cycle arrest and death in all the PCa cell lines. These effects were evident also in cells maintained under hypoxic conditions to resemble the in vivo situation more closely.

CONCLUSIONS

In conclusion, 1) PCa cells exploit GS and extracellular Asn to adapt to Gln depletion under conditions mimicking the in vivo environment; 2) this adaptive mechanism is not detectable in conventional media; 3) growth and survival of both androgen-dependent and -independent PCa cells are effectively suppressed by combining shortage of extracellular Gln and Asn with the inhibition of Glutamine Synthetase.
Acknowledgment: GT is supported by Fondazione Umberto Veronesi, Italy

P006

CBX3 AS A PROGNOSTIC BIOMARKER IN LUNG CANCER

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BACKGROUND-AIM

Lung cancer remains one of the most prevalent and aggressive malignancies worldwide, characterized by poor prognosis and high mortality rates. As the understanding of the molecular underpinnings of lung cancer evolves, the focus has shifted towards chromatin-modifying proteins, which play pivotal roles in gene expression regulation and cellular processes. Chromobox protein 3 (CBX3), has garnered attention due to its potential involvement in oncogenesis. In lung cancer, CBX3 plays an epigenetic role in enhancing tumor growth and progression. he main objectives of this study involve the molecular and functional characterization of CBX3 in lung cancer; the role of possible CBX3 modulators to check how they affect lung cancer; understanding the gene expression profile analysis to characterize CBX3 function in lung cancer

METHODS

• In silico data analysis on gene and epigenetic profiling from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression portal (GTEx).
• Immunohistochemical analysis using a lung cancer tissue microarray containing 120 lung cancer tissue samples.

RESULTS

In silico data indicate high expression of CBX3 in lung cancer tissues associated with a worse prognosis. Notably, RNA sequencing data from TCGA and GTEx show that lung tissues express CBX3 at a significantly higher level than normal lung tissues. The survival analysis with the TCGA database between samples with low and high expression levels of CBX3 indicates that high CBX3 expression is significantly associated with shorter overall survival inlung cancer patients. Immunohistochemical analysis using a lung cancer tissue microarray containing 120 lung cancer tissue samples indicate that CBX3 protein expression in lung tissues is mainly nuclear. Furthermore, CBX3 expression is strongly associated with tumor grade, clinical stage, tumor dimension, tissue type and pathology diagnosis. This clarifies the role of CBX3 in the development of lung cancer by observing that it is part of the molecular machinery that promotes aggressiveness in other ways.

CONCLUSIONS

Elucidating the role of CBX3 in lung cancer progression provides valuable insights into the molecular mechanisms driving tumor aggressiveness. The identification of CBX3 as a potential biomarker establishes a new avenue for targeted therapies, which could be particularly beneficial in the treatment of patients with advanced lung cancer.

P007

DEVELOPMENT AND CHARACTERIZATION OF A NOVEL FLUORESCENT AN-TI-CEACAM5 ANTIBODY FOR ENHANCED IMAGE-GUIDED SURGERY IN GASTRIC CANCER

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BACKGROUND-AIM

Gastric cancer (GC) is the third leading cause of cancer death globally, with 1 million new cases estimated each year. Surgery is the main therapy, but currently there are no specific methods to distinguish malignant from healthy tissue and to discriminate between metastatic and non-metastatic lymph nodes; therefore, the need to resect all lymph nodes indiscriminately increases the intraoperative risks for patients.
Contrast agents enable real-time evaluation of the surgical field using both conventional laparoscopy systems and the robotic platforms such as the da Vinci. However, their effectiveness is limited by a lack of specificity, highlighting the urgent need for targeting tumor cell-specific biomarkers. This study aims to develop an innovative fluorescence tool to be used in real time surgical procedure for enhancing metastatic mapping during robotic gastrectomy.

METHODS

We examined the expression of the most GC markers, including carcinoembryonic antigen cell adhesion molecule-5 (CEACAM5) and Claudin-4 (CLDN4), in GC cell lines using western blotting. To further evaluate their expression, we performed immunohistochemistry (IHC) on tumor and healthy tissue samples from 30 GC patients who underwent partial gastrectomy at the Digestive System Surgery Unit, AOU Careggi, Florence (Ethics Committee authorization number 27004_bio). Additionally, we validated anti-CEACAM5 expression on patient-derived organoids. Furthermore, we developed a fluorescent molecule targeting CEACAM5 on the surface of GC cells and assessed its binding properties on patient tissue slices.

RESULTS

Our analysis revealed that CLDN4 and CEACAM5 were exclusively expressed in tumor cell lines, both non-malignant and metastatic, while they were absent in the healthy gastric cell line used as a control. IHC results showed that while CLDN4 was strongly expressed in both tumor and healthy tissues, CEACAM5 was significantly present in tumor samples but absent or minimally expressed in healthy mucosa ($p=0.0018$). These findings confirmed CEACAM5 as a more specific tumor marker. The validation of CEACAM5 expression on patient-derived organoids further supported its specificity for tumor tissue. Additionally, the fluorescent molecule designed to target CEACAM5 selectively bound to GC patient tissue slices, confirming its tumor specificity.

CONCLUSIONS

In the future, our system could be an excellent tool to be tested in-vivo and then applied to precision surgery during robotic gastrectomy procedures.

P008

ONCOSTATIN M MODULATES TUMOR-STROMA INTERACTIONS AND FUELS INTRAHEPATIC CHOLANGIOCARCINOMA PROGRESSION VIA JAK/STAT SIGNALLING

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BACKGROUND-AIM

Intrahepatic cholangiocarcinoma (iCCA)) is a highly aggressive and chemoresistant tumor with poor prognosis. Oncostatin M (OSM), a member of the IL-6 cytokine family, modulates the tumor microenvironment (TME) toward an immunosuppressive phenotype orchestrating the crosstalk between cancer and stromal cells. Particularly, OSM-mediated signalling pathways (mainly via p-STAT3) have been associated with proliferation, angiogenesis, epithelial-mesenchymal transition (EMT), metastasis and maintenance of cancer stem cells (CSCs). Since the role of OSM in iCCA has been only partially investigated, this project aims at elucidating the involvement of this factor and its receptor in iCCA biology and tumor-stroma interactions.

METHODS

OSM and its receptors expression were analyzed in human iCCA samples via immunohistochemistry and RT-PCR. Two human iCCA cell lines (HuCCT-1, CCLP-1), patient-derived organoids (PDOs) and two stromal cell types (CAFs and THP-1-derived macrophages) were used. Cell motility was evaluated by chemotaxis and invasion assays. Protein expression was determined by Western blotting. OSMR and gp130 knockdown were performed using siRNA. Conditioned media (CM) from OSM-treated iCCA cells were used to treat CAFs and THP-1-derived macrophages. RNA sequencing was performed on iCCA cells cultured on 3D matrix scaffolds, treated with OSM and the JAK1/2 inhibitor ruxolitinib.

RESULTS

iCCA cell lines and PDOs expressed both OSM and OSMR. In human iCCA specimens, OSM and OSMR were overexpressed in tumor cells compared to peritumoral tissue. OSM induced a dose-dependent increase in iCCA cell migration and invasion, associated with cytoskeletal remodeling and EMT induction. These effects were reduced by OSMR or gp130 silencing, or by pre-treatment with ruxolitinib. CM from OSM-treated iCCA cells increased CAF migration, suggesting a role for OSM in promoting a fibrotic and tumor-supportive microenvironment. Moreover, CM also promoted M2-like polarization in THP-1-derived macrophages, as indicated by the increased expression of M2 markers (TGFβ, IL-10, CCL22). Transcriptomic analysis revealed a JAK-dependent up-regulation of mesenchymal signature genes and down-regulation of immune-related pathways, along with a JAK-independent activation of Wnt signalling.

CONCLUSIONS

This study identifies the OSM/OSMR axis as a novel system potentially implicated in cholangiocarcinogenesis with pro-tumorigenic effects and modulation of the tumor microenvironment.

P009

IDENTIFICATION OF AN ERK5/HYPOXIA INDUCIBLE FACTOR 1 α INTERPLAY IN INTRAHEPATIC CHOLANGIOCARCINOMA

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BACKGROUND-AIM

Cholangiocarcinoma (CCA) is the second most common liver cancer after hepatocellular carcinoma, and constitutes a heterogeneous group of malignancies that arise at any point of the biliary tree. CCA is characterized by poor prognosis and limited therapeutic options. Recently, our group reported the importance of the mitogen-activated protein kinase extracellular signal regulated kinase 5 (ERK5) in supporting the survival and proliferation of intrahepatic CCA (iCCA) cells both in vitro and in vivo. To identify additional molecular targets in CCA, we investigated the possible functional relationship between ERK5 and hypoxia inducible factors α, the main regulators of the response to hypoxia, a condition that is typical of the tumour microenvironment.

METHODS

Intrahepatic CCA cell lines (CCLP-1 and HUCCT-1) were grown at different time points under normoxia and hypoxia. Gene silencing was performed with short harpin RNA for ERK5 gene (MAPK7). Protein expression analysis was investigated by Western Blotting. For the pharmacological treatments, HIF and ERK5 inhibitors (HIFi and ERK5i) effects were evaluated in term of cell viability using MTT and trypan blue exclusion assays, in term of 3D tumor growth and live dead cell assay using Calcein AM/PI on spheroids.

RESULTS

We found that ERK5 phosphorylation and HIF1α expression are increased in hypoxia, and increased activity of the latter was confirmed by the consistent increase of the target genes, Carbonic Anhydrase 9 (CAIX) and Glucose transporter 1 (GLUT1). We also identified a functional relationship between ERK5 and HIF-1α. Indeed, genetic and pharmacologic inhibition of ERK5 prevented the hypoxia-induced increase of CAIX and GLUT1. Moreover, combined treatments of ERK5i and HIFi resulted to be more effective than single treatments in reducing cell viability and 3D spheroid growth in CCA cell lines. Moreover, quantification of dead cells in CCA spheroids showed that the combination of ERK5i and HIFi determined an increase of the percentage of PI positive cells with respect to controls.

CONCLUSIONS

Overall, these results highlighted a functional interaction between ERK5 and HIF-1α in regulating CCA homeostasis, pointing to a novel potential therapeutic strategy for the treatment of CCA.

P010

FRUCTOSE WORSENS HEPATIC STEATOSIS AND INFLAMMATION BY RESHAPING MACROPHAGE FUNCTIONAL CAPACITIES

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BACKGROUND-AIM

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common liver disease worldwide. MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH), characterized by cellular injury and parenchymal inflammation, with or without fibrosis. Multiple factors drive the transition from MASLD to MASH, but the current view considers chronic inflammation an essential trigger. Consolidated evidence indicates that overconsumption of foods rich in fats and simple sugars such as fructose contributes to hepatic inflammation in MASLD/MASH by inducing lipo-toxicity, oxidative stress and gut dysbiosis. However, little is known about the role of fructose in aggravating MASLD/MASH by directly modulating inflammatory cell functions. In this study, we investigated the capacity of fructose to reshape macrophage functional properties by in vivo and in vitro approaches.

METHODS

C57BL/6 wild-type (WT) mice received a high-fat diet (HFD) with or without 30% fructose (w/v) in drinking water for up to 16 weeks. RAW 264.7 macrophage cell line was cultured in DMEM medium with or without fructose (50mM) for 48 hours.

RESULTS

We observed that fructose supplementation exacerbated hepatic steatosis and increased the hepatic expression of pro-inflammatory/pro-fibrogenic markers (e.g., CCl2, TNF-α, IL-15, OPN and Gal-3) in mice. It also promoted a significant expansion of the hepatic pool of monocyte-derived macrophages (MoMFs) associated with an enhanced recruitment of CD8+ cytotoxic T-lymphocytes. Of note, the fructose in vitro stimulation of RAW 264.7 macrophage cell line induced the expression of lipogenic genes (i.e. SREBP1) and increased the cytoplasmic content of neutral lipids. Furthermore, fructose stimulated the production and secretion of critical mediators already found over-expressed in MASH livers, such as IL-15 and OPN and increased the antigen-presentation capabilities of RAW 264.7 cells. The analysis of transcriptional factors revealed that fructose significantly promoted only the interferon regulatory factor (IRF) – 4 expression in RAW 264.7 cells.

CONCLUSIONS

These results indicate that fructose contributes to MASLD/MASH progression by reshaping macrophage functional capacities through the induction of molecular pathways driven by IRF-4.

P011

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

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BACKGROUND-AIM

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.

METHODS

AgNPs were synthesized and characterized through spectroscopy UV-vis sprectroscopy, FTRI and NanoSight. Moreover, electron transmitted 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.

RESULTS

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 disfunction 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.

CONCLUSIONS

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.

P012

THE ICOS/ICOSL AXIS: A NOVEL TARGET FOR MASH-RELATED HCC?

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BACKGROUND-AIM

Metabolic-associated steatohepatitis (MASH) can progress to hepatocellular carcinoma (MASH-HCC), one of the most common forms of primary liver cancer. The pathogenesis of MASH-HCC involves chronic inflammatory responses, driven by interactions between macrophages and activated T cells. Although the precise mechanisms sustaining MASH-associated liver inflammation and its progression to fibrosis and HCC remain incompletely understood, recent studies have demonstrated that the costimulatory molecule ICOS and its ligand ICOSL play key roles in mediating hepatic inflammation and fibrosis in MASH. These findings suggest a potential role for the ICOS/ICOSL axis in the transition from MASH to HCC.

METHODS

The role of the ICOS/ICOSL axis was investigated in C57BL/6 wild-type (WT), ICOS- and ICOSL-knockout (KO) mice subjected to a MASH-related hepatocarcinogenesis model. A subset of mice received a recombinant form of ICOS (ICOS-Fc) during the last eight weeks of treatment.

RESULTS

All WT mice developed multiple tumor nodules. In contrast, approximately 14% of KO animals showed no visible tumors, and those that developed HCC exhibited significantly fewer and smaller tumor masses compared to WT mice. This reduction was accompanied by significant downregulation of the FOXP3 transcription factor and a marked expansion of $CD8^+$ cytotoxic T cells in both KO groups. Additionally, the expression of pro-survival mediators such as amphiregulin (AREG) was significantly reduced in these mice. Biochemical and morphological analyses also revealed decreased activation of the phospho-ERK1/2 pathway and reduced Ki67 expression, as assessed by western blot and immunohistochemistry (IHC), respectively. Notably, ICOS-Fc administration exacerbated tumor burden in both WT and ICOS KO mice, while having no effect in ICOSL KO mice, as expected. Specifically, ICOS-Fc treatment induced the expression of anti-apoptotic mediators such as Bcl-XL and reduced the levels of cytotoxic T cell-associated molecules, including CD8 and CXCR6, in WT mice. In ICOS KO animals, ICOS-Fc further increased AREG expression.

CONCLUSIONS

Disruption of the ICOS/ICOSL axis attenuated tumor development in mice by reducing regulatory T cell populations, expanding $CD8^+$ cytotoxic T lymphocytes, and downregulating signaling pathways involved in cell proliferation and survival. Overall, these findings support the potential of targeting the ICOS/ICOSL pathway as a novel therapeutic strategy to counteract MASH-associated HCC.

P013

EXPLAINING THE DYNAMICS OF TUMOR AGGRESSIVENESS: AT THE CROSSROAD BETWEEN BIOLOGY AND ARTIFICIAL INTELLIGENCE

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer, lacking targeted therapies and associated with poor prognosis. A key challenge in personalized medicine is the accurate prediction of metastatic risk to guide treatment strategies. Traditional approaches based on immune microenvironment classification often fail to adequately stratify patients with high metastatic potential.

METHODS

We developed ARIADNE, a computational strategy based on a Boolean network model of the epithelial-mesenchymal transition (EMT) pathway. Transcriptomic data fromRNA-Seq TNBC patients are mapped onto the network to identify hybrid epithelial/mesenchymal phenotypes associated with tumor aggressiveness. ARIADNE's predictive capability was validated using three independent TNBC patient cohorts. Moreover we have investigated ARIADNE as predictive tool in patients treated with immunotherapy in HER2 negative breast cancer patients. Finally we developed a cytokine score, derived from the average expression of 23 cytokine genes, measured both in bulk and single-cell data.

RESULTS

ARIADNE successfully stratified TNBC patients by metastatic risk, identifying phenotypic subtypes linked to disease progression. Notably, the method detected a high-risk group with elevated immune markers that were misclassified by immune microenvironment-based strategies. In addition, ARIADNE demonstrated prognostic value in predicting responses to neoadjuvant chemotherapy, offering clinically relevant insights beyond conventional classification methods. We also showed that the cytokine score in combination with ARIADNE enhances predictive resolution for immunotherapy response.

CONCLUSIONS

Collectively, these studies underscore the potential of ARIADNE, especially when integrated with immune profiling, to guide personalized immunotherapeutic strategies in breast cancer and beyond, contributing to more precise and individualized oncology care.

P014

MARINE-DERIVED ANTRAQUINONE INDUCES MITOCHONDRIA IMPAIRMENT IN MELANOMA CELL LINE.

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BACKGROUND-AIM

Melanoma is one of the most aggressive cancers due to its high metastatic potential and mortality rate. Furthermore, many patients develop drug resistances to common therapeutic compounds, underscoring the need to discover new anticancer agents. Natural products, derived from both terrestrial and marine organisms, represent a fundamental reservoir of bioactive compounds for drug development, especially in the area of cancer therapies. Among these, Hallacrome (HC), a marine-derived anthraquinone isolated from the mucus of polychaete Halla parthenopeia, has shown positive results in ecotoxicity tests and antimicrobial activity. Therefore, aim of the study was to explore the biological effects of HC on the human A375 melanoma cell line.

METHODS

Cell growth in human A375 melanoma cell line was evaluated for 48 hours following the treatment with 8.4 μM HC. To investigate the proteomic profile, LC-MS/MS was performed after 4 hours exposure to HC. Mitochondrial respiration was assessed by measuring oxygen consumption rate (OCR) using Seahorse XFe96 Analyzer. Mitochondrial morphology and membrane potential were evaluated using MitoView Green and JC-1, respectively. Reactive oxygen species (ROS) were measured using CM-H2DCFDA.

RESULTS

Cells treated with HC exhibited a significant reduction of cell growth compared to untreated cells and this effect was observable as early as 4 hours post-treatment. Proteomic analysis highlighted that HC altered proteins were mainly involved in mitochondrial organization. Functional assays performed on mitochondrial activity demonstrated that HC modulates OCR in a manner closely resembling the effects of FCCP (carbonylcyanide-p-trifluoromethoxyphenylhydrazone), a known mitochondrial uncoupler that disrupts ATP synthesis by interfering with the proton gradient. Furthermore, decreased mitochondrial activity induced by HC led to an altered production of intracellular ROS.

CONCLUSIONS

Given the central role of mitochondria in cancer metabolism and progression, the ability of HC to target mitochondrial bioenergetics highlights its potential as a candidate for anticancer therapy.
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P015

NRF2-DRIVEN PROLIFERATION AND METABOLIC REPROGRAMMING IN NORMAL HEPATOCYTES

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BACKGROUND-AIM

Cancer cells undergo a metabolic reprogramming to maintain the redox homeostasis and to support their increased bioenergetic and biosynthetic needs. This metabolic shift, mainly characterized by impaired oxidative phosphorylation (OXPHOS) and increased glucose utilization, is driven by the Keap1-Nrf2 system, one of the crucial regulators of cellular proliferation and intracellular oxidative stress. Indeed, Nrf2 promotes tumor progression through the up-regulation of pentose phosphate pathway (PPP). Both metabolic reprogramming and Nrf2 activation characterize hepatocellular carcinoma (HCC), one of the most lethal human cancers worldwide. Despite the increasing interest in this complex pathway, no studies have been performed to elucidate whether, during proliferation, also normal hepatocytes can activate a metabolic reprogramming similar to that described in neoplastic cells.

METHODS

To answer this question, hepatocyte proliferation was induced in vivo and in vitro by primary mitogens: lead nitrate (LN) or triiodothyronine (T3). The effect of genetic deletion of Nrf2, as well as molecular and functional metabolic changes, were evaluated through metabolome, histochemical, enzymatic, gene expression and protein analyses.

RESULTS

In both in vitro and in vivo approaches, only LN exposure induced the activation of Nrf2-target genes and well-established features of cancer metabolic reprogramming, such as enhanced glycolysis and oxidative PPP, and reduced OXPHOS in non-tumorigenic hepatocytes. Contrarywise, NRF2 ablation suppressed the LN-induced metabolic reprogramming, while no metabolic changes were observed in proliferating hepatocytes subjected to T3.

CONCLUSIONS

Hepatocytes exposed to LN undergo a NRF2-dependent metabolic reprogramming, characterized by NRF2 activation, in both in vitro and in vivo studies. Importantly, LN administration offers a rapid and useful model to investigate the metabolic changes characterizing normal hepatocytes, and to dissect the differences and similarities in metabolic reprogramming in normal and neoplastic hepatocytes.

P016

THE ROLE OF NEW RESEARCH PARAMETERS OF THE BC 6800 PLUS ANALYZER IN THE DIFFERENTIAL DIAGNOSIS OF LYMPHOID NEOPLASMS

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BACKGROUND-AIM

A precise and standardized classification system in hematology is essential. The latest edition of the WHO classification emphasizes the importance of the complete blood count (CBC) and the assessment of cell morphology in peripheral blood and bone marrow smears for diagnosing lymphoid neoplasms. This study aimed to evaluate the diagnostic role of Research Use Only (RUO) parameters from the BC-6800 Plus Mindray analyzer in differentiating chronic lymphocytic leukaemia (CLL), acute lymphoblastic leukaemia (ALL), and other lymphoproliferative disorders (LPD), to develop new diagnostic algorithms to improve the sensitivity and specificity of CBCs in diagnosing lymphoid neoplasms.

METHODS

In this retrospective study, a complete blood count (CBC) was performed in 90 patients (M: F 66:34%, median age 67 years) admitted at the emergency department of Novara's Hospital with a pathological blood count (ALL, n=14; CLL, n=47; other LPD, n=29). The association of basic (WBC, Hb, RDW, NE#, LY#, MO#, PLT) and research cell parameters (NLR or NE/LY ratio, NMR or NE/MO ratio, LMR or LY/MO ratio, NeuX, NeuY, NeuZ, LymX, LymY, LymZ, MonX, MonY, MonZ) was evaluated by univariable and multivariable logistic regression.

RESULTS

In multivariable analysis, Hb (p=0.02), NeuY (p=0.04), MonY (p=0.01), were found to be independent predictors of CLL compared to ALL. This multivariable model correctly classified 93.4% of cases with an AUC of 0.91 (95%CI 0.81-1.0). Independent predictors of CLL comparing to other LPD patients were MO# (p=0.003), LymY (p<0.0001) and MonY (p=0.004). This multivariable model correctly classified 77.6% of cases with an AUC of 0.86 (95%CI 0.78-0.95). For the comparison between ALL and other LPD patients, NeuZ (p=0.01) and NeuY (p=0.04), were identified as independent predictors. This model correctly classified 93% of cases with an AUC of 0.98 (95%CI 0.95-1.0).

CONCLUSIONS

The utilization of morphological research parameters may provide valuable help, without additional costs, in the early diagnosis of ALL, CLL and other LPD.

P017

MULTIOMICS PROFILING REVEALS A SUBSET OF NKG2A+ Vδ2+ T CELLS ASSOCIATED WITH RESPONSE TO PD-1 BLOCKADE IN NON-SMALL CELL LUNG CANCER

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BACKGROUND-AIM

Lung cancer represents the leading cause of cancer-related mortality on a global scale and ranks among the most prevalent malignant epithelial neoplasms, surpassed only by hormone-dependent tumors in incidence. Non-small cell lung cancer (NSCLC) constitutes approximately 85% of all lung cancer cases. The aim of this study was to characterize γδ T lymphocytes within the tumor microenvironment of NSCLC, assessing their frequency and function in both tumor tissue and peripheral blood. Additionally, we analyzed the phenotypic and transcriptomic changes of these cells before and after treatment with PD-1 inhibitors.

METHODS

An initial bioinformatic analysis of single-cell RNA sequencing data was performed to identify distinct subsets of γδ T lymphocytes. These findings were subsequently validated ex vivo through flow cytometry, assessing the frequency and immunophenotypic profile of γδ T cells in the peripheral blood of NSCLC patients, both before and after treatment with anti-PD-1 antibodies.

RESULTS

Our analysis revealed a distinct cluster of γδ T lymphocytes expressing NKG2A, likely capable of exerting significant cytotoxic activity in peripheral tissues. This subset was found to be significantly more abundant in healthy tissues than in tumor tissues and exhibited a notable increase in the peripheral blood of NSCLC patients following checkpoint inhibitor immunotherapy.

CONCLUSIONS

These findings suggest that γδ T lymphocytes, particularly the NKG2A-expressing subset, may contribute substantially to the antitumor immune response and play a role in modulating tumor progression in NSCLC. Moreover, preliminary flow cytometric data indicate that quantification of circulating NKG2A# γδ T cells during therapeutic follow-up could represent a promising and minimally invasive biomarker for predicting clinical response to immune checkpoint inhibitors.

P018

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

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BACKGROUND-AIM

Acute myeloid leukemia (AML) is one of the deadliest hematologic cancers in adults, with less than 30% of patients surviving beyond five years due to drug resistance and relapse. This underscores the urgent need for novel molecular targets and therapeutic strategies. AML is characterized by disrupted hematopoietic differentiation, where neoplastic clones fail to mature into fully differentiated blood cells. Recent studies have highlighted the role of m6A RNA methylation and its regulators as critical drivers in AML and other cancers. While METTL3, the primary m6A methyltransferase, has been shown to promote leukemogenesis, the function of other METTL family members, including METTL16, remains poorly understood.
This study aims to investigate the role of METTL16 in AML. We will explore whether METTL16 contributes to AML progression and identify the molecular pathways involved. Additionally, we will assess the potential of targeting METTL16 to inhibit AML cell proliferation, providing a new avenue for therapeutic intervention.

METHODS

We adopted a reverse genetic lentiviral-based approach to explore the role of METTL16 in acute myeloid leukemia (AML) cell lines and in ovo. We evaluated the phenotypic effect of METTL16 knockdown (KD) and analyzed transcriptomic and epitranscriptomic perturbations in shMETTL16 cells by RNA-seq and methylated RNA immunoprecipitation and sequencing (MeRIP-seq). Further, we carried out RNA immunoprecipitation (RIP), Actinomycin D, Western Blot (WB), co-immunoprecipitation (Co-IP), Real-Time qPCR, and ChIP assays to confirm the involvement of METTL16 to regulate MXD4 stability and consequently MYC activity.

RESULTS

Our investigation revealed that METTL16 is overexpressed in AML samples. Genetic depletion or pharmacological inhibition of METTL16 strongly affected the growth strongly affected the growth 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, essential for leukemogenesis. Strikingly, suppression of MXD4 rescued the expression levels of MYC genes restoring AML cell survival.

CONCLUSIONS

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

P019

ANTITUMORAL EFFICACY OF AUNRS-LADEN ECFCS IN VITRO AND IN VIVO: DECODING THE HEAT AND RAYS COMBO TREATMENT IN BREAST CANCER AND MELANOMA CELLS

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BACKGROUND-AIM

Radiotherapy remains a cornerstone in metastatic cancer treatment but is often hindered by tumor hypoxia and radioresistance. Gold nanorods (AuNRs) offer promise in enhancing radiotherapy through hyperthermia, yet their clinical impact is limited by poor tumor targeting. Building on our previous findings demonstrating the tumor-homing ability of Endothelial Colony Forming Cells (ECFCs) loaded with AuNRs, this study advances their use as a biologically targeted delivery system for precise radiotherapy enhancement.

METHODS

Using 3D in vitro tumor models and in vivo studies with nude rats, we demonstrate that ECFCs actively home to hypoxic tumor regions, overcoming traditional nanoparticle delivery limitations. This targeted approach ensures efficient AuNR accumulation, enhancing photothermal activation and maximizing radiosensitization

RESULTS

In vitro, ECFC-loaded AuNRs significantly amplify radiotherapy effects, inducing ferroptosis in melanoma and inhibiting autophagy in breast cancer cells—revealing distinct tumor-specific mechanisms. Moreover, ECFC-AuNRs suppress tumor proliferation and angiogenesis, blocking vessel-like structure formation in vitro and in vivo.

CONCLUSIONS

By integrating cellular therapy with nanotechnology, this study presents a novel strategy to counter radioresistance and improve therapeutic precision. These findings lay the foundation for a clinically viable, patient-specific approach, unlocking new possibilities in advanced cancer treatment

P020

LONG PENTRAXIN 3 (PTX3) AS A REGULATOR OF LYMPHANGIOGENESIS AND LYMPHOGENOUS DISSEMINATION IN MELANOMA

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BACKGROUND-AIM

Melanoma is one of the most aggressive forms of cutaneous tumor, being responsible for 90% of skin cancer-related death each year. Melanoma-associated lymphangiogenesis plays a pivotal role in tumor dissemination from the primary site to the draining lymph nodes (LNs) and then to distant organs, turning melanoma into a life-threatening cancer. The long pentraxin-3 (PTX3) exerts pleiotropic functions in physiopathological conditions, including cancer, where it has been observed to act as a tumor suppressor. We have previously observed that PTX3 impairs melanoma growth and its invasive/metastatic features by affecting several aspects of cancer progression, but to date little is known about its role in tumor-associated lymphangiogenesis.

METHODS

Here, we treated lymphatic endothelial cells (LECs) with lymphangiogenic stimuli and melanoma conditioned media, and their effect on PTX3 expression was assessed in vitro through qPCR and western blot analysis. The effect of both recombinant PTX3 and its endogenous overexpression on the activation of LECs was evaluated in terms of proliferation, migration and sprout formation. RNAseq analysis was performed on PTX3-overexpressing LECs. Furthermore, in vivo matrigel plug assay and lymphatic dissemination of melanoma cells were performed in mice characterized by lymphatic expression of PTX3.

RESULTS

This work sheds light on the regulatory role of PTX3 in lymphangiogenesis and in melanoma lymphogenous dissemination. Preliminary observations conducted on human skin biopsies show that PTX3 is downregulated in lymphatic vessels (LVs) of primary melanoma specimens compared to normal skin. Accordingly, we observed that both lymphangiogenic and melanoma-derived factors downregulate PTX3 expression in LECs, and that both treatment with recombinant PTX3 and its endogenous/genetic overexpression reduce LEC activation. Furthermore, we observed that in a transgenic mouse model, lymphatic expression of PTX3 hampers lymphangiogenesis in vivo and significantly reduces the metastatic spreading of melanoma to the draining LN in two different cell dissemination models in mice.

CONCLUSIONS

In conclusion, our data highlight an inhibitory role of PTX3 in melanoma-associated lymphangiogenesis, its downregulation in LECs representing a pivotal step in lymphogenous dissemination of melanoma. Thus, lymphatic PTX3 may have mechanistic, prognostic and therapeutic implications in melanoma.

P021

MICROBIOTA AND AI IN THE SERVICE OF PRECISION PATHOLOGY

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BACKGROUND-AIM

The human microbiota, the complex ecosystem of bacteria, viruses, fungi, and other microorganisms inhabiting our bodies, is increasingly recognized as a key determinant of health and disease. Precision medicine, which tailors diagnosis and treatment to individual patient characteristics, is now integrating microbiota analysis to develop personalized therapeutic strategies. However, the vast interindividual variability and complexity of host-microbiota interactions demand advanced analytical approaches to translate this data into clinically actionable insights.

METHODS

Artificial intelligence (AI) has emerged as a transformative tool in this field, capable of processing large, multivariate datasets and uncovering hidden patterns. Machine learning and deep learning algorithms are being employed to correlate specific microbial profiles with diseases, predict treatment responses (e.g., to antibiotics, probiotics, or dietary interventions), and optimize targeted therapies such as fecal microbiota transplantation. AI also enables the integration of multi-omics data (metagenomics, metatranscriptomics, metabolomics) with clinical information, improving patient stratification and deepening our understanding of disease mechanisms.

RESULTS

We have applied the AI-Microbiota combination for infection and disease prevention through microbial signatures, nutritional and immune regimens for metabolic and infectious diseases, and microbiota modulation to enhance cancer immunotherapy efficacy.

CONCLUSIONS

The convergence of microbiota research and AI is opening new frontiers in precision medicine, positioning the microbiota as a dynamic "therapeutic organ." Fully realizing this potential will require collaboration among pathologists, microbiologists, clinicians, and bioinformaticians alongside regulatory frameworks to ensure the safety, reliability, and equity of these technologies.

P022

XENOBIOTIC RECEPTORS/MICROBIOTA CROSSTALK AT MUCOSAL SURFACES

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BACKGROUND-AIM

Xenobiotic receptors, like the pregnane X receptor (PXR) and aryl hydrocarbon receptor (AHR), play a significant role in regulating the body's response to foreign compounds (xenobiotics) including drugs, environmental toxins, and microbial metabolites at mucosal surfaces. These receptors, found in tissues like the gut and lungs, help maintain homeostasis by sensing and responding to xenobiotics, often through interactions with the local microbiota. A reciprocal regulation between these two receptors has been recently suggested but the exact mechanism is still poorly understood.

METHODS

We have investigated the role and cross-talk between PXR and AHR in vivo in murine models of pulmonary infections with Aspergillus fumigatus and Pseudomonas aeruginosa in PXR- or AHR-deficient mice.

RESULTS

Despite the low expression level of PXR in the lung, PXR-deficient mice were resistant to both A. fumigatus and P. aeruginosa infections. Histopathological analysis showed no or very low level of inflammation in the lung, confirmed by the low expression level of NLRP3 gene, pro-inflammatory cytokines and reduced neutrophil recruitment. AHR levels were elevated in PXR-deficient mice and likely mediated the increased resistance to infections via a fine regulation of NLRP3. It appears that both PXR and AhR likely contribute to the antimicrobial immune response in the lung either independently or via a fine regulation of the NLRP3 inflammasome.

CONCLUSIONS

Our study suggests that the role of PXR and AHR may go beyond the xenobiotics metabolism to include a fine regulation of the inflammatory response at the host/microbiota interface via a mutual cross-talk.

P023

UVI5008, THE FIRST SELECTIVE, REVERSIBLE, NON-COVALENT BRUTON'S TYROSINE KINASE EPI-INHIBITOR FOR B-CELL MALIGNANCIES

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BACKGROUND-AIM

Aberrant BCR signaling via BTK drives B-cell malignancies, including CLL. Although covalent BTK inhibitors (BTKis) like ibrutinib have significantly improved clinical outcomes, treatment resistance due to mutations in BTK (e.g., C481S) or downstream effectors such as PLCγ2 remains a major clinical challenge. Recently, non-covalent BTKis (e.g., pirtobrutinib) have shown promising results, but the therapeutic resistance and relapse still occur. This study discovered and characterized a novel and compelling alternative therapeutic strategy to address the urgent need for more effective treatments in B-cell malignancies resistant to conventional BTK inhibitors.

METHODS

Kinase activity screening was performed on UVI5008, followed by computational docking and QSAR analysis. The findings were subsequently validated through a comprehensive set of in vitro and ex vivo assays, including enzymatic, cellular, transcriptional, genetic, epigenetic and genomic assays, on CLL patient-derived PBMC and cell lines, as well as in vivo studies using a genetically engineered mice model.

RESULTS

In this study, we identify a novel tyrosine kinase inhibitory activity of UVI5008, which, to date, stands as the only known epigenetic modulator (epi-inhibitor) that directly targets BTK, influencing both its expression and enzymatic functions. Our comprehensive analysis, spanning in silico, in vitro, ex vivo, and in vivo approaches reveals that UVI5008 effectively inhibits both wild-type BTK and the C481S mutated isoform, commonly associated with BTK inhibitor resistance in CLL. Treatment with UVI5008 in B-cell lymphoma and leukemia disorders led to a substantial increase in cellular apoptosis, accompanied by a notable reduction in BTK protein levels and phosphorylation, and attenuation downstream signaling, demonstrating superior efficacy compared to Ibrutinib. Additionally, ex vivo treatment of patient-derived CLL samples and murine models corroborated these results, further supporting the potential of UVI5008 as a promising therapeutic agent.

CONCLUSIONS

UVI5008 represents a promising pharmacological alternative to current BTK inhibitors. As the first-in-class selective, reversible, and non-covalent BTK epi-inhibitor, it demonstrates potent and durable efficacy in relapsed/refractory CLL, including cases harboring the challenging C481S BTK mutation, addressing a critical clinical gap in current treatment option.

P024

POLYSTYRENE NANOPLASTICS ALTER THE EXPRESSION OF GLUTAMINE-RELATED ENZYMES IN MESENCHYMAL STROMAL CELLS: AN ANTI-OSTEOGENIC EFFECT

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BACKGROUND-AIM

Plastic pollution is a subject of global alarm. In the environment, plastics break into micro- (MPs) and nanoplastics (NPs), which enter the food chain, potentially impacting human health. Once internalized, NPs have been found to induce oxidative stress in several kinds of human cells. Among its several metabolic activities, the amino acid glutamine (Gln) plays an anti-oxidant role. Indeed, Gln-derived glutamate is used by the xCT exchanger to take up cystine, needed for glutathione synthesis once reduced to cysteine. Moreover, Gln also has a pro-osteogenic activity, promoting the osteoblastic differentiation of bone marrow mesenchymal stromal cells (MSCs) by fueling the synthesis of asparagine (Asn) via the enzyme asparagine synthetase (ASNS). This study investigates the effects of NPs on human MSC differentiation and Gln metabolism.

METHODS

Human bone marrow MSCs (primary cells and the immortalized line hTERT-MSC) were treated with commercially available fluorescent polystyrene NPs (50 nm diameter, 10-200 μg/ml). NP-treated and control cells were then incubated in osteogenic or adipogenic media, and the expression of differentiation markers was checked by RT-PCR. NPs uptake was assessed by confocal microscopy and amino acid content through LC-MS.

RESULTS

MSCs internalize NPs that are widely distributed in the cytoplasm with a preferential localization in lysosomes, which result markedly enlarged in exposed cells. Even at the highest dose, NPs do not affect MSC viability, yet they trigger oxidative stress, as demonstrated by lowered levels of glutathione, HMOX1 induction and increase in xCT transporter activity. Although Gln, glutamate and Asn contents remain unchanged, NPs lower ASNS expression while induce Glutamine Synthetase, a metabolic phenotype associated with anti-osteogenic effects. Indeed, NPs dose-dependently decrease the expression of osteogenic differentiation markers (ALPL, SPARC, COL1A1, ASNS, GLS, SLC38A2), while slightly increase those linked to adipogenesis (PPARG, LEP, ADIPOQ, FABP4).

CONCLUSIONS

In conclusion, polystyrene NPs enter MSCs, where they cause oxidative stress and dysregulate Gln metabolism, impairing the osteoblastic differentiation of MSCs. Possible links with osteoporosis and bone disease remain to be established.

P025

SOMATIC FOCALLY AMPLIFIED SUPER-ENHANCERS AS NOVEL ONCOGENIC DRIVER IN NEUROBLASTOMA

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BACKGROUND-AIM

Neuroblastoma (NB) is a pediatric cancer with poor prognosis, often driven by somatic focal amplifications (FAs) that enhance the expression of oncogenes, including MYCN and ALK. Emerging evidence suggests that non-coding FAs can involve super-enhancers (SEs), controlling cell identity and contributing to tumor development. However, the specific role of SEs within focal amplifications (FA-SEs) in NB remains poorly understood. In our study we hypothesize that FA-SEs drive tumorigenesis by upregulating critical oncogenes and influencing disease progression. Our goal is to identify NB-specific FA-SEs and clarify their impact on tumor biology and clinical outcomes.

METHODS

To identify NB-specific FA-SEs, we integrated whole-genome sequencing (WGS) data from NB patients with public NB-specific SE datasets. Associations between FA-SEs and clinical features were assessed using Fisher's test. FA-SE targets were predicted through analysis of capture Hi-C data from SK-N-BE(2)-C, SH-SY5Y, SH-EP, and GI-ME-N cell lines, along with matched RNA-seq and H3K27ac ChIP-seq data from 60 NB samples. Predicted interactions were validated by ChIP-seq and RT-PCR in NBL-S and SK-N-AS cells following treatment with enhancer-modulating compounds (ORY-1001, GSK2879552, GSK-LSD1, A485, and JQ1).

RESULTS

Analysis of WGS from 136 NB patients identified four FA-SEs located at cytobands 11q13.3 and 17q25.3, which were significantly associated with adverse prognostic markers. These FA-SEs were subsequently validated in an independent cohort of 180 patients. Key oncogenes, including CCND1, FGF19, IGHMBP2, LTO1, MRGPRD, MYEOV, and TPCN2, were identified as targets of 11q13.3 FA-SEs, while CANT1, DNAH17, TK1, and BIRC5 were linked to 17q25.3 FA-SEs. Functional studies revealed that epi-pharmacological modulation of enhancer activity, specifically through compounds altering H3K27ac and H3K4me1 histone marks, resulted in consistent changes in the expression of FA-SE target genes. Ongoing validation using CRISPR-Cas9-based epigenome editing aims to confirm direct enhancer–target gene interactions and further elucidate the functional significance of these regulatory elements.

CONCLUSIONS

Our findings demonstrate that somatic FAs altering super-enhancers drive oncogenes expression, contributing to NB progression and poor clinical outcomes. FA-SEs emerge as powerful biomarkers for patient stratification and prognosis and represent novel therapeutic targets in neuroblastoma.

P026

FUNCTIONAL AND COGNITIVE FRAILTY ASSESSMENT IN AGED MICE: TOWARDS STANDARDIZED AND TRANSLATIONAL PRECLINICAL METRICS

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BACKGROUND-AIM

Frailty is a multidimensional condition involving both physical and cognitive domains, with critical relevance for translational aging research. Standardized functional and cognitive assessments in preclinical models are essential to evaluate geroscience-based interventions and to bridge the gap between animal studies and clinical applications. We report the development and validation of two complementary approaches in natu-rally aging mice: a Cognitive Frailty Index (CoFI) and composite grip strength scores, designed to provide ro-bust, feasible, and standardized measures of age-related decline.

METHODS

All the measurements were derived from the analysis of large cohorts of aged C57BL/6J mice followed for until the end of their lifespan. The CoFI integrates multiple parameters from longitudinal Barnes Maze testing to generate a single cognitive frailty metric, adjusted for physical performance where needed. Grip strength was evaluated using five different methods (two weight lift tests, cage lift test, grip strength meter with trapeze bar and grid), and novel composite scores were derived to balance robustness and feasibility. Correlations with age, mortality, muscle size, and sex differences were analyzed.

RESULTS

The CoFI strongly correlated with age and mortality, providing good discrimination between short- and long-lived mice, and allowed separation of cognitive and physical components of frailty. Grip strength methods correlated negatively with age and muscle size, with the Grip Strength Meter (trapeze bar) methods showing the strongest associations. The composite scores preserved high correlation with age while reducing testing time, enhancing their suitability for large-scale studies. Importantly, normalization of grip strength to body weight introduced bias in aged mice, favoring the use of absolute values.

CONCLUSIONS

Our integrated functional and cognitive assessment framework offers a practical and standardized approach for preclinical frailty evaluation in aged mice. These metrics support robust longitudinal monitoring and could facilitate the testing of geroscience interventions targeting functional decline and cognitive impair-ment. Future studies will explore integration with automated cage monitoring systems to further enhance translational value.

P027

JAK2-V617F MUTATION SCREENING IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS, CALABRIA REGION (SOUTHERN ITALY), YEAR 2024

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BACKGROUND-AIM

The Philadelphia negative myeloproliferative neoplasms (MPNs), included polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF) are chronic myeloproliferative diseases caused by clonal expansion of malignant hematopoietic stem/progenitor cells. JAK2 is a tyrosine kinase and V617F is an acquired mutation, occurring in 95% of PV, 55% of ET, and 65% of MF patients. Identification of the presence of the JAK2-V617F mutation is fundamental for diagnosing, prognoses, and monitoring of patients with chronic MPNs and its detection has been included in the World Health Organization (WHO) guidelines.

METHODS

JAK2 V617F mutation screening was requested, between 1 January 2024 to 31 December 2024, for 303 patients (68% male, mean age 60.3±14.0 years; 32% female, mean age 62.0±16.0 years) by the medical staff of the "Annunziata Hospital", Cosenza (CS), Southern Italy and by outpatient physicians present across the Calabria region. The analysis was performed by semi-quantitative Real-time PCR and Ipsogen JAK2 MutaQuant kit using DNA extracted from peripheral blood samples.

RESULTS

The mutation was identified in 77 (25%) of the 303 patients analyzed. Of the total number of requests, 46% of positive patients (62% M) were referred from hospital departments, whereas 12% (50% M) originated from outpatient physicians. MF patients had the highest average age. Hospital departments with the highest requests for JAK-mutational screening were the Hematology Unit, followed by the Department of Transfusion Medicine of our institution.

CONCLUSIONS

The prevalent diagnosis, in the analyzed sample of Calabrian patients carrying the JAK2 V617F mutation, was TE, followed by PV and MF, in agreement with the current literature. From the standpoint of clinical appropriateness, requests originating from hospital clinicians were more appropriate (46% of positive patients) compared with those from outpatient physicians (12% positivity). These results highlight the importance of adequate training of all healthcare personnel, in particular those who work outside the hospital setting, to optimize costs and improve the efficiency of healthcare services.

P028

IDENTIFICATION OF A RAS FAMILY MEMBER AT THE CROSSROAD BETWEEN ERK5 AND ERK1/2 MAPK PATHWAYS IN MELANOMA

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BACKGROUND-AIM

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 improved survival, especially when combined with immunotherapy. However, resistance mechanisms frequently limit their efficacy. Studies suggest that ERK1/2 inhibition may result in ERK5 activation, likely as a compensatory mechanism to support tumor growth. This work investigated the role of a small Ras-family GTPase (here referred to as X-Ras) in the cross-regulation of ERK1/2 and ERK5 pathways in BRAFV600E-mutant melanoma.

METHODS

BRAFV600E-mutated A375 and Sk-Mel-5 melanoma cells were silenced for ERK5 using specific shRNAs, or treated with non-targeting shRNA (shNT) as control. X-Ras was silenced using siRNAs, with non-targeting siRNA (siNT) as control. mRNA expression was analyzed by qRT-PCR. Small-molecule inhibitors used included ERK5 inhibitors (JWG-071, XMD8-92), MEK1/2 inhibitor (Trametinib), BRAF inhibitor (Vemurafenib) and ERK1/2 inhibitor (SCH-772984).

RESULTS

Genetic and pharmacological inhibition of ERK5 elicits the increase of MEK1/2 and ERK1/2 phosphorylation and of X-Ras expression in melanoma cells. Silencing X-Ras prevents this compensatory activation and enhances the anti-proliferative and pro-apoptotic effects of ERK5 inhibition. Interestingly, X-Ras is similarly responsible for ERK5 activation after BRAF-MEK1/2-ERK1/2 pathway inhibition by combined vemurafenib/trametinib or SCH-772984 treatments. X-Ras knockdown prevents ERK5 phosphorylation induced by the inhibition of BRAF (Vemurafenib and Trametinib) or ERK1/2 (SCH-772984). Moreover, combined inhibition of X-Ras and BRAF-MEK1/2-ERK1/2 pathway is more effective in reducing melanoma cell viability than single treatments, pointing to a resistance/compensatory mechanism exerted by X-RAS upon ERK5 activation.

CONCLUSIONS

X-Ras emerges as the tipping point for the reciprocal compensatory activation of ERK1/2 and ERK5, and its inhibition potentiates the effectiveness of treatments targeting individual pathways.

P029

DEVELOPMENT OF A PANCREATIC CANCER DRUG-NANOCARRIER SYSTEM SELECTIVELY TARGETING TUMOUR CELLS AND TUMOUR STROMA VIA THE HERG1–INTEGRIN β1 COMPLEX TO OVERCOME TREATMENT FAILURE

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BACKGROUND-AIM

Pancreatic cancer treatment is limited by poor drug delivery due to dense, fibrotic stroma. The tumor microenvironment, rich in CAFs, immune cells, and ECM, forms a barrier that hinders therapy. Key interactions via integrin complexes like hERG1/β1, found in cancer cells, offer a specific target. We aim to design a delivery system that overcomes stromal barriers to selectively reach tumor cells.

METHODS

To target the hERG1/β1 integrin complex, we generated a bispecific antibody specifically recognizing the heteromeric structure of the complex. The antibody was used in vitro assays using pancreatic cancer cell lines, where its specificity, binding affinity, and internalization capabilities were assessed via immunofluorescence and immunohistochemistry analysis. In vivo experiments was performed using orthotopic murine models of pancreatic cancer, evaluating volume, stiffness tumor and in vivo imaging. Then, we initiated the conjugation of the anti-hERG1/β1 antibody with gemcitabine-loaded nanoparticles to develop a targeted drug delivery system.

RESULTS

In vitro studies demonstrated that the scDb-hERG1-β1 antibody specifically binds to PDAC cell lines expressing the hERG1/β1 complex, reducing PANC-1 cell viability and migratory capacity. PDAC tumor samples collected at AOUC were IHC-positive for the complex, and tissue expression significantly correlated with its presence in patient blood. An orthotopic PDAC mouse model was established via ultrasound-guided injection of PANC-1 and RLT-PSC cells at different ratios. Tumors in the 1:5 group showed increased hypoxia and reduced perfusion, suggesting that stellate cells alter the tumor microenvironment. Histology revealed more uniform collagen distribution in co-injected tumors, and RT-qPCR confirmed increased ECM component expression. The antibody was produced at scale by the UNIFI team (5 mg in PBS), validated, packaged, and shipped for nanoparticle conjugation. The resulting complexes retained binding ability (peptide and cell ELISA). A second 5 mg batch in Tris-HCl buffer was produced to improve stability and facilitate further conjugation steps, while preserving cytotoxic activity.

CONCLUSIONS

The scDb-hERG1-β1 antibody shows promise for PDAC therapy by targeting tumor cells and microenvironment. Future work will focus on in vivo testing and optimizing nanoparticle conjugates for clinical use.

P030

IMPACT OF Aβ OLIGOMERS ON HUMAN ASTROCYTES DERIVED FROM HEALTHY INDIVIDUALS AND ALZHEIMER'S PATIENTS

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BACKGROUND-AIM

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, amyloid-β (Aβ) plaque accumulation and neurofibrillary tangles. Small Aβ oligomeric species have pronounced neurotoxic and pro-inflammatory effects. Astrocytes, essentials for central nervous system homeostasis, show functional impairment in AD, contributing to disease progression. In this study, we investigated the effects of $A\beta_{1-42}$ oligomers on primary human astrocytes from healthy individuals (H) and AD patients (AD) of different sexes.

METHODS

Cells were treated with $A\beta_{1-42}$ oligomers at a concentration of 10 μM (monomer equivalents). The intracellular calcium level was analyzed by flow cytometry at 48 and 72 hours, labelling the cells with Fluo-4-AM dye. At the same time points, we assessed mitochondrial membrane potential using the JC-1 assay and the proteasomal activity with a Proteasome Activity Assay kit. Cellular viability was detected by MTT assay, and an Annexin V/7AAD flow cytometry assay detected apoptotic cells. The senescent phenotype was evaluated after 120 hours by analyzing several senescent markers.

RESULTS

We demonstrated that astrocytes from all individuals can internalize $A\beta_{1-42}$ oligomers, particularly in females. After internalization, proteasome activity increased in H astrocytes but decreased in AD astrocytes. Exposure to $A\beta_{1-42}$ oligomers disrupted calcium homeostasis and impaired mitochondrial membrane potential in astrocytes from all subjects. This treatment reduced cell viability by inducing apoptosis in H astrocytes; however, the surviving cells exhibited a reactive phenotype characterized by increased proliferation and secretion of neuroinflammatory mediators. In contrast, $A\beta_{1-42}$ treatment induced a senescent state in AD astrocytes, especially in those from female patients, as evidenced by relevant markers: elevated β-galactosidase activity, upregulation of $p14^{ARF}$, and the presence of senescence-associated heterochromatin foci.

CONCLUSIONS

Our findings reveal distinct responses of H and AD astrocytes to $A\beta_{1-42}$ treatment, highlighting the close relationship between cellular senescence and Alzheimer's disease. This suggests a possible role for cell senescence in the pathogenesis and progression of the disease, especially in women.

P031

T CELLS, B CELLS AND NANOMEDICINE: THE IMMUNE CELL ACTIVATION PROJECT ON THE INTERNATIONAL SPACE STATION

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BACKGROUND-AIM

T cells and B cells are crucial cells for the adaptive specific defence of the host. The European Space Agency (ESA) Project "Immune Cell Activation (ICA)" was developed to understand how the space environment, and in particular microgravity, would affect the activation of T cells and B cells by ferromagnetic nanoparticles and A375 melanoma cells. The working hypothesis was to develop novel therapeutic (nano) tools for specific targeting of superficial cutaneous cancers such as melanoma.

METHODS

Basically, our approach combines the benefits of immunology (such as selectivity of the targets) and nanotechnologies in order to use nano-engineered Cytotoxic T cells (CTL) as 'Trojan horse' against melanoma. This technique allows nanoparticles embedded in immune cells, to arrive at the site of the disease, even when injected systemically, undisturbed by uptake by the immune system. The activation of T cells, B cells by nanoparticles and melanoma cells was performed in the KUBIK hardware on the International Space Station (ISS).

RESULTS

By performing our experiments in the KUBIK on the ISS we found that:
In nano-engineered Cytotoxic T cells specific for melanoma cells a relevant increase of interferon gamma (IFN-gamma) and tumour necrosis factor alpha (TNF-alpha), essential for cytotoxic activity against tumours. B cells produced high levels of IgG and IgM. In A375 melanoma cells a significant ($p < 0.05$) differential gene expression in a proliferation related set of genes that encode for proteins related to DNA replication, DNA topologic change, DNA repair (TOP2A, RNASEH1, EXO1), to cell cycle (CKS1B, CCNB2, MAD2L2), to mitosis (NUSAP1) were less expressed in the microgravity environment.

CONCLUSIONS

All these results suggest that Nanoparticles up-load in microgravity could improve the immune cells activation and their toxic effector function on melanoma target cells. Nanoparticles up-load in microgravity could also decrease the cancer cell proliferation which is one of the basic mechanisms of cancer progression.

P032

T3 REVERTS METABOLIC REPROGRAMMING IN A MASH-INDUCED MODEL OF HCC

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BACKGROUND-AIM

Hepatocellular carcinoma (HCC), the most common primary liver malignancy, is characterized by profound metabolic reprogramming that promotes aggressive growth, survival, and therapy resistance. Recent evidence has highlighted the antineoplastic potential of the thyroid hormone triiodothyronine (T3) in a chemically induced rat model (R-H) of hepatocarcinogenesis, suggesting promising new therapeutic avenues. However, while chemically induced models have greatly advanced our understanding of hepatocarcinogenesis, they fail to fully recapitulate the multifactorial complexity of MASLD/MASH-related HCC. To address this, we investigated the effects of T3 in a MASH-induced nutritional model of hepatic carcinogenesis, which more accurately reflects the metabolic and inflammatory features of the human disease.

METHODS

Male F344 rats were subjected to an experimental model consisting of a single dose of diethylnitrosamine (DENA) followed by a choline-devoid methionine-deficient (CMD) diet for 4 months. The CMD diet was then replaced with a basal diet and 10 months after DENA animals were randomly divided into two groups: the first group was maintained in basal diet whereas the second group was exposed to 5 cycles of T3-supplemented diet. At the end of the last cycle, all animals were sacrificed. Tumors and surrounding tissue for each group were laser-microdissected and subjected to RNA-seq.

RESULTS

CMD-induced HCCs were marked by a metabolic shift toward anaerobic glycolysis, characteristic of the Warburg phenotype, associated with increased oxidative stress and a concomitant activation of antioxidant defense mechanisms, compared to the surrounding tissue. On the contrary, T3 was able to completely revert this phenotype as demonstrated by the increase in oxidative phosphorylation (OXPHOS) activity accompanied by a reduction in antioxidant defenses.

CONCLUSIONS

These findings highlight the ability of T3 to counteract the metabolic reprogramming typical of hepatocellular carcinomas induced by MASH. The activation of the T3-dependent intracellular pathways, by restoring oxidative metabolism and reducing the reliance on antioxidant defenses, also in advanced neoplastic lesions, could represent a potential antineoplastic strategy.

P033

TARGETING THE ESCAPE: SETD8 INHIBITION ENHANCES TEMOZOLOMIDE EFFICACY IN MGMT-METHYLATED GLIOBLASTOMA

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BACKGROUND-AIM

Glioblastomas IDH wt (GBs) w are among the most therapeutically challenging brain tumors and are associated with a particularly poor prognosis. One of the key prognostic biomarkers in this context is the methylation status of the MGMT promoter, which predicts a better response to the alkylating agent temozolomide (TMZ). However, even in MGMT-methylated patients, clinical outcomes remain dismal.
In a recent study (Della Monica et al., Cell Death and Disease, 2023), we reported that a lysine methyltransferase, SETD8, is overexpressed in a significant subset of glioblastomas and that its expression correlates with poorer prognosis. SETD8 is involved in multiple physiological and pathological processes, with its main function being the monomethylation of histone H4 on lysine 20 (H4K20me1), a modification essential for DNA damage repair. We focused our study on the evaluation of H4K20me1 epigenetic modification function in GBs.

METHODS

Experiments were performed in GB cell lines and primary cells. Cell viability and death were assessed by MTT and caspase activity assays. UNC0379-induced vesicles were investigated by immunofluorescence and western blot. UNC0379 transcriptional targets were investigated by Real time PCR and Nascent RNA Capture Kit. SETD8 transcriptional targets were validated by ChIP and ChIP-Seq.

RESULTS

We found that this epigenetic modification occurs at the promoter of the p62 gene, which plays a pivotal role in autophagy flux, in glioblastoma cells. This histone modification is associated with repression of p62 gene expression. When we antagonised SETD8 activity using a chemical inhibitor, aberrant transcription of p62 occurred.
A high level of p62 seems to be associated with a block in autophagic flux.
Based on this, we hypothesise that combining TMZ treatment with a SETD8 inhibitor would be effective, as TMZ not only induces DNA damage but also activates autophagy as a cellular survival mechanism. Therefore, inhibiting SETD8 may disrupt this pro-survival response, potentially limiting the emergence of TMZ-resistant clones.

CONCLUSIONS

Indeed, experiments using this combination therapy demonstrated increased cell death in MGMT-methylated GBM cells, as well as a reduction in the number of TMZ-resistant clones when the co-treatment was administered chronically.duce autophagic failure.

P034

LACTYLATION-DRIVEN REGULATION OF CA IX EXPRESSION IN CHEMORESISTANT GASTRIC CANCER

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BACKGROUND-AIM

Chemoresistance in gastric adenocarcinoma is frequently associated with metabolic reprogramming, characterized by enhanced glycolysis and lactate accumulation. Recent studies have highlighted lactylation, a lactate-dependent post-translational modification, as an emerging mechanism of gene regulation. We investigated whether lactate-induced histone-lactylation modulates the expression of carbonic anhydrase IX (CA IX), a known hypoxia-inducible enzyme, and its role in chemoresistance.

METHODS

Gastric cancer cell lines (AGS, ACC-201) were chronically treated with FLOT (5-Fluorouracil, Oxaliplatin and Docetaxel) chemotherapy to generate resistant sublines (FLOTr). Metabolic profiling was performed using Seahorse analysis and lactate quantification in the conditioned media. CA IX expression was assessed by flow cytometry after treatment with lactate or 2-deoxy-D-glucose (2-DG). HIF-1α expression was analyzed by immunohistochemistry, western blot, and immunofluorescence under normoxia, hypoxia, and hypoxia with or without the HIF-1 inhibitor acriflavine.

RESULTS

CA IX is known to be regulated by HIF-1α under hypoxic conditions. However, in normoxia, FLOTr cells showed elevated CA IX expression despite undetectable HIF-1α levels, as confirmed in both ACC-201 and AGS models, and, noteworthy, in patient-derived FFPE samples. This uncoupling suggests alternative regulatory pathways. Metabolic analysis revealed increased glycolytic flux and lactate accumulation in FLOTr cells. Exogenous lactate administration upregulated CA IX levels, while glycolysis inhibition through 2-DG treatment reduced its expression. These findings support a HIF-independent mechanism of CA IX regulation, likely involving lactate-driven histone lactylation that may be responsible for CA IX promoter accessibility and enhanced transcription, linking metabolic rewiring to epigenetic control in chemoresistant gastric cancer.

CONCLUSIONS

Our data suggest that lactylation functions as a key link between altered tumor metabolism and CA IX expression, promoting chemoresistance in gastric cancer. Targeting this HIF-independent metabolism-driven pathway may open novel therapeutic avenues.

P035

PBMC ACTIVATION AND TUMOR KILLING ENHANCED BY SLC-0111 AND FLOT COMBINATION THERAPY

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BACKGROUND-AIM

Chemoresistance and immune evasion are major challenges in gastric cancer (GC) treatment. Tumor acidosis and carbonic anhydrase IX (CA IX) overexpression contribute to both. SLC-0111, a selective CA IX inhibitor, may enhance chemotherapy efficacy and modulate the immune microenvironment. We investigated its effects in combination with FLOT (5-Fluorouracil, Oxaliplatin and Docetaxel) on GC spheroids and patient-derived organoids (gcPDOs), focusing on peripheral blood mononuclear cell (PBMC) activation and cytotoxicity.

METHODS

GC spheroids were treated for 7 days with FLOT, SLC-0111, or both, with or without PBMCs from healthy donors. Cell viability was assessed via Calcein-AM/PI staining and spheroid volume analysis. PBMC activation and cytotoxic markers (CD3+CD8+ T cells, perforin, granzyme B) were analyzed by flow cytometry and TRAIL by qPCR. Drug response was further tested on gcPDOs, and serum CA IX levels were analyzed in responder vs. non-responder patients.

RESULTS

Calcein/PI staining revealed a marked increase in cell death in AGS WT spheroids treated with the combination of SLC-0111 and FLOT with respect to the treatment alone. Notably, the cytotoxic effect was significantly amplified in the presence of PBMCs, as shown by intense PI staining and reduced spheroid volume. In untreated controls, spheroids remained largely viable, with minimal PI uptake. This indicates that PBMCs are not passive but actively enhance tumor killing when properly stimulated. Flow cytometry confirmed that PBMCs exposed to the combination treatment displayed increased activation of CD3+CD8+ T cells and elevated expression of perforin, granzyme B, and TRAIL, evaluated in qPCR, consistent with induction of immunogenic cell death (ICD). In gcPDOs from a non-responder patient, SLC-0111 selectively inhibited tumor proliferation. Serum analysis showed higher CA IX levels in FLOT non-responders.

CONCLUSIONS

SLC-0111 boosts FLOT efficacy not only through direct tumor cell killing but also by enhancing PBMC-mediated immune responses. These findings support CA IX as a therapeutic target and potential biomarker for chemoresistance and immune modulation. Combining CA IX inhibitors with chemo-immunotherapy warrants further clinical evaluation.

P036

CONDITIONED MEDIA DERIVED FROM HEPG2 CELLS TREATED WITH PHOSPHOELEGANIN SYNTHETIC-DERIVED COMPOUND PE/2 IMPROVE METABOLIC FUNCTION IN 3T3-L1 ADIPOCYTES

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BACKGROUND-AIM

We have previously demonstrated that the phosphoeleganin synthetic-derived compound PE/2 acts as an insulin-sensitizing agent, reducing interleukin 6 (IL6) levels and enhancing insulin signaling in hepatocellular carcinoma cell lines (HepG2). Since secreted liver proteins, including IL6, can affect insulin sensitivity in various organs, such as adipose tissue, we evaluated the effect of conditioned media (CM) from HepG2 cells on the insulin pathway in differentiated 3T3-L1 adipocytes.

METHODS

Proteins and mRNA levels from differentiated 3T3-L1 adipocytes treated with CM were analyzed by Western blot and Real-Time RT-PCR, respectively.

RESULTS

HepG2 CM containing insulin (CM-Ins) stimulated the phosphorylation of the insulin receptor (INSR) and AKT by 1.16- and 1.24-fold, respectively, compared to the CM, although these results were not statistically significant. Interestingly, CM from HepG2 treated with PE/2 and containing insulin (PE/2 CM-Ins) increased INSR and Akt phosphorylation by 2.13- and 1.82-fold, respectively, when compared to CM, and 1.83- and 1.47-fold compared to CM-Ins. Total levels of INSR and AKT did not change in the presence of CM-Ins and PE/2 CM-Ins. Furthermore, in the presence of PE/2 CM-Ins, the mRNA levels of adiponectin were 1.87- and 1.58-fold higher than those of CM and CM-Ins, respectively, while TNFα expression remained unchanged.

CONCLUSIONS

Although preliminary, the data obtained suggest that PE/2 CM-Ins significantly enhances insulin signaling and increases adiponectin levels compared to CM alone and CM-Ins in 3T3L1 adipocytes. Since adiponectin serves as an insulin sensitizer, it can be hypothesized that conditioned media from HepG2 cells treated with PE/2, by raising adiponectin, may improve insulin signaling in adipocytes.

P037

THE KDM2A-NOTCH3 AXIS AS DRIVER OF STEMNESS AND EPITHELIAL-MESENCHYMAL TRANSITION IN HEPATOCELLULAR CARCINOMA

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BACKGROUND-AIM

Notch3 receptor is frequently upregulated in hepatocellular carcinoma (HCC) and associated with poor prognosis. Emerging evidence implicates KDM2A, a histone demethylase, in promoting oncogenesis via epigenetic regulation. Our study investigates whether KDM2A modulates Notch3 expression in HCC, with a focus on its potential role in stemness and epithelial-mesenchymal transition (EMT).

METHODS

KDM2A and Notch3 knockdown (KD) Huh7 HCC cells were established. Quantitative PCR and immunohistochemistry (IHC) were used to evaluate mRNA and protein expression in HCC samples of different grades. Transcriptomic profiling and migration assays were performed to evaluate functional consequences. EMT and stemness marker were analyzed by immunoblotting and qPCR in both KDM2A-KD and Notch3-KD Huh7 compared to wild type cells.

RESULTS

IHC analysis revealed that both Notch3 and KDM2A were elevated in HCC compared to peritumoral tissue, with expression correlating with tumor grade ($p<0.001$ for KDM2A). Notch3 positivity was observed in CD34+ vessels in grade 1 (G1) and in invasive hepatocytes in G3 tumors. KDM2A KD led to a reduction in Notch3 mRNA and protein levels ($p<0.0001$ and $p<0.01$). Aberrant expression of the stemness markers CD44, CD133, and EpCAM was detected in both KDM2A-KD cells ($p<0.05$, $p<0.001$, and $p<0.0001$, respectively) and Notch3-KD cells ($p<0.0001$ for all markers). Notch3 KD altered the expression of EMT-related genes, including Vimentin ($p<0.001$), β-catenin ($p<0.05$), E-cadherin ($p<0.05$), Slug ($p<0.05$), Zeb1 ($p<0.05$), Snail1 ($p<0.01$), Twist ($p<0.0001$), and MMP9 ($p<0.001$). VEGF protein levels were also reduced following Notch3-KD ($p<0.05$), consistent with IHC findings. Loss of Notch3 impaired cell migration ($p<0.05$) and altered the EMT transcriptomic profile ($p<0.01$).

CONCLUSIONS

Our data suggest a functional KDM2A-Notch3 axis in HCC, where KDM2A sustains Notch3 expression, contributing to stemness maintenance and EMT activation. Notch3 localization in tumor vessels and hepatocytes indicates its role in angiogenesis and invasiveness. Notch3 silencing is shown to affect EMT regulators, reinforcing its significance in HCC progression. These findings highlight the KDM2A-Notch3 axis as a promising candidate for the development of novel targeted therapies.

P038

PRO-DIFFERENTIATION TREATMENT OF LUNG CANCER CELLS SIGNIFICANTLY REDUCES CANCER STEM CELL AND PATHOGENIC TRAITS IN VITRO

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BACKGROUND-AIM

Cancer Stem Cells (CSCs) are linked to tumour relapse after therapy due to aggressiveness, chemoresistance and metastatic potential. Recent reports suggest pro-differentiation approaches may be successful in leukemia and solid cancer models, for instance through conversion of mammary carcinoma cells into post-mitotic adipocytes, offering a possible route to enhancing current anti-proliferative therapies.

METHODS

In the present study, the non-small cell lung cancer model A549 was exposed to medium supplemented with serum and pro-adipogenic factors (DM), compared to control standard medium (SM). Effects on the cell proliferation, migration and adhesion properties were assessed in vitro through Crystal Violet staining, cell cycle FACS analysis, wound healing assay and iCELLigence technology, respectively. Gene and protein expression levels of CSC markers, aquaporins and autophagy-related genes were evaluated through RT-qPCR, immunofluorescence analysis and Western Blot, while ALDH1 activity was analysed by flow cytometry.

RESULTS

7-day exposure of A549 cells to DM induced drastic changes in phenotype, with significant increase in cellular footprint and vesicle accumulation. Increased Surfactant Protein C expression and alkaline phosphatase activity, two alveolar markers, alongside the accumulation of multilamellar vesicles confirmed through TEM imaging, suggested a differentiation response upon DM treatment. These results aligned with a decrease in CSC markers, including ABCG2 expression and ALDH1 activity, and coincided with significant inhibition of cell proliferation and migration, while cell adhesion properties increased. DM treatment also caused a significant reduction in CSC features such as clonogenic ability (2D), anchorage-independent colony formation (soft agar culture) and spheroid growth (3D), alongside lowered aquaporin function.

CONCLUSIONS

Overall, these results suggest that pro-differentiation medium reduces A549 lung cancer cell pathogenicity by upregulating alveolar lineage markers while strongly decreasing CSC features, thus representing a valuable strategy for further preclinical testing.

P039

CONTRIBUTION OF CONCURRENT RB1 AND P53 PATHWAYS ALTERATION TO THE DEVELOPMENT OF THE PRIMITIVE NEURONAL COMPONENT IN GLIOBLASTOMA

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BACKGROUND-AIM

Glioblastoma with Primitive Neuronal Component (GBM-PNC) represents a GBM variant characterised by conventional GBM areas along with immature early neuronal components. We recently reported that RB1 and TP53 pathway alterations represents GBM-PNC molecular hallmarks and PNC phenotype relies on expression of EBF3, an early neurogenic transcription factor, directly controlled by the MYC transcription factors in accessible chromatin sites. We established in vitro models to test if p53 and RB1 pathway signalling impairment is required to commit conventional GBMs toward a GBM-PNC phenotype.

METHODS

We selected RB1-wildtype patient-derived glioma stem cells (GSCs) and created a CRISPR/Cas9 RB1 knockout model with either loss or retention of p53 functionality. RB1-mutated GBM-PNC GSC line was transduced with lentiviral vector to test if RB1 ectopic expression could revert the GBM-PNC phenotype.

RESULTS

Interestingly, both the cell cycle and the cytogenetic analysis highlighted an increased tetraploidy in the double-mutants as compared to the mocks and the transcriptomic data confirmed the upregulation of cell cycle phase transition pathways and of mitotic spindle assembly checkpoints. By a drugs high-throughput screening, we demonstrated that double mutants are more resistant to cell cycle inhibitors. This aggressiveness was observed also by their increased sensitivity, early detection and repair of DNA damages upon radiation. Given the GBM-PNC refractoriness to the standard regimen for conventional GBMs, we are further investigating double mutant-sensitive hit compounds. The immunopathological staining of GSC-derived organoids showed that the double-mutants markedly reduced the expression of GFAP glial marker and sustained that of βIII-tubulin, maintaining an early neuroectodermal signature. Moreover, they presented multinucleated cells with hyperchromatic nuclei, mirroring the aberrant mitotic features of the PNC component of tumours, but without expressing EBF3. We assessed that the EBF3 promoter was methylated, suggesting an epigenetic status that might prevent the gain of a full biphasic phenotype. The failed ectopic expression of RB1 in the GBM-PNC GSCs might reinforce this hypothesis.

CONCLUSIONS

We demonstrated that RB1 loss alone is not sufficient to trigger the switch from conventional GBM into GBM-PNC. Thus, concomitant p53 and RB1 pathways alterations represent a predisposing feature and additional epigenetic status will be necessary to obtain a full GBM-PNC phenotype.

P040

PLURIPOTENCY ASSOCIATED ENHANCER RNA CONTROLS NANOG GENE EXPRESSION IN MOUSE EMBRYONIC STEM CELLS

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BACKGROUND-AIM

The role of enhancers in the three-dimensional regulation of gene expression has been extensively characterized. However, the functional relevance of enhancer-derived RNAs (eRNAs), transcribed from these regulatory elements, remains poorly understood. In mouse embryonic stem cells, the Nanog locus constitutes a representative model for studying tridimensional epigenetic regulation in stemness. This locus presents three super-enhancers, located approximately -5 kb, -45 kb, and +60 kb relative to the Nanog transcription start site, all of which are transcriptionally active and establish physical interactions with the Nanog promoter. Notably, despite these shared structural features, the individual contributions of each super-enhancer to Nanog transcription exhibit distinct regulatory effects.

METHODS

mESCs were cultured in serum/LIF conditions. The -5 kb eRNA was identified by strand-specific cDNA synthesis. Its depletion was performed via RNA interference. DNA methylation was analyzed by bisulfite sequencing, histone modifications by ChIP-qPCR, and chromatin looping by 3C assays. RNA immunoprecipitation (RIP) was used to detect interactions between the eRNA and Rad21, a component of the cohesin complex.

RESULTS

We identified a distinct eRNA transcribed from the -5 kb Nanog enhancer. Knock down analysis revealed its essential role in preserving an active epigenetic state at the Nanog locus. Depletion of the eRNA induced DNA methylation, chromatin remodeling, and loss of enhancer-promoter looping, leading to Nanog silencing and spontaneous endodermal differentiation. Notably, exogenous re-expression of Nanog failed to rescue the original chromatin structure or looping, indicating a unique upstream role for the eRNA. Mechanistically, the eRNA directly interacts with Rad21, suggesting it regulates chromatin topology via cohesin recruitment.

CONCLUSIONS

Our findings uncover the -5 kb eRNA as a critical regulatory element that maintains the Nanog locus in a transcriptionally competent state. By influencing chromatin architecture and interacting with cohesin, this eRNA safeguards pluripotency in mESCs. Its broader role in disease contexts involving Nanog dysregulation warrants further investigation.

P041

PROMOTION OF MITOCHONDRIAL FUSION TRIGGERS ANTI-TUMOR ACTIVITY AND ENHANCES VENETOCLAX SENSITIVITY IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Increasing evidence indicates that unbalanced mitochondrial dynamics contributes to tumor onset and progression. In this study, we investigated the functional role of the mitochondrial E3 ubiquitin ligase, Membrane Associated Ring-CH-Type Finger 5 (MARCH5)—a negative regulator of mitochondrial fusion—in multiple myeloma (MM) cell growth, metabolic reprogramming, and drug resistance.

METHODS

Mitochondrial morphology was assessed by transmission electron microscopy (TEM). Cell viability was measured using the CellTiter-Glo assay. MARCH5 overexpression was achieved using lentiviral vectors, while gene silencing was performed using siRNAs. Transcriptomic profiling was conducted using the Illumina NGS platform. mRNA and protein expression levels were quantified by quantitative RT-PCR and Western blot analysis, respectively. In vivo anti-tumor efficacy was evaluated in NOD-SCID mice engrafted with MM cells. Mitochondrial oxygen consumption rate (OCR) was measured using high-resolution respirometry.

RESULTS

March5 expression was upregulated, particularly in bortezomib-resistant MM cells. Silencing March5 promoted mitochondrial fusion, as evidenced by elongated mitochondria and increased MFN2 protein levels. Conversely, March5 overexpression (OE) reduced MFN2 stability through enhanced ubiquitylation. Genetic or pharmacological induction of MFN2 using leflunomide showed both in vitro and in vivo anti-myeloma effects. RNA-seq of March5-depleted cells revealed downregulation of oxidative phosphorylation (OXPHOS) pathways linked to venetoclax resistance. Accordingly, both March5 silencing and MFN2 induction enhanced venetoclax sensitivity, without synergizing with other standard therapies. Metabolically, March5 silencing or venetoclax reduced OXPHOS, whereas March5 OE increased it, counteracting venetoclax-induced metabolic stress.

CONCLUSIONS

These data indicate that promoting mitochondrial fusion induces metabolic perturbations that lead to anti-myeloma activity and enhance sensitivity to venetoclax.

P042

Aβ-INDUCED SENESCENCE IN ALZHEIMER'S ASTROCYTES DRIVES NEURONAL DEATH VIA SASP AND METABOLIC DYSREGULATION

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BACKGROUND-AIM

Aging is the main risk factor for Alzheimer's disease (AD). One hypothesis links its onset to the accumulation of soluble $A\beta_{1-42}$ oligomers, which are neurotoxic and also affect astrocytes, key regulators of brain homeostasis. Under chronic stress or aging, astrocytes can undergo senescence, acquiring a senescence-associated secretory phenotype (SASP) and metabolic alterations that compromise neuronal viability. We investigated the SASP profile and metabolic enzyme expression in primary human astrocytes from AD patients (AD) and healthy subjects (HS) of both sexes after $A\beta_{1-42}$ oligomers exposure. We also assessed the effect of astrocyte conditioned medium (CM) on differentiated SH-SY5Y cells.

METHODS

AD and HS astrocytes were exposed to $A\beta_{1-42}$ oligomers and analyzed at 120h for senescence (WB), SASP (Quantibody array), and metabolism-related genes (RT-PCR). CM from treated astrocytes was applied to SH-SY5Y cells for 24h, then viability and apoptosis were measured by MTT assay and Annexin V/7AAD staining.

RESULTS

$A\beta_{1-42}$ oligomers induced a senescent phenotype in AD astrocytes, particularly in females, while a subset of HS astrocytes underwent apoptosis; the surviving HS astrocytes developed a reactive and hyperproliferative phenotype. SASP analysis revealed that both AD and HS astrocytes show inflammatory responses to $A\beta_{1-42}$, but follow distinct inflammatory and neurotrophic trajectories. Metabolic profiling showed that $A\beta_{1-42}$-treated AD female astrocytes had decreased LDH-A and MCT4 expression, with increased HK2 and G6PD, compared to controls and $A\beta_{1-42}$-treated AD males. These data suggest enhanced glycolytic activity but impaired lactate production and export in AD female astrocytes, whereas these functions increased in AD males. Notably, only CM from $A\beta_{1-42}$-treated AD-astrocytes induced apoptosis in SH-SY5Y cells, suggesting a specific neurotoxic role.

CONCLUSIONS

Although SASP factor levels in senescent AD-astrocytes were not as elevated as expected, their CM still caused SH-SY5Y cell death. Metabolic changes observed after $A\beta_{1-42}$ treatment suggest that factors beyond classical SASP components may contribute to neuronal damage. These effects may reflect the distinct inflammatory and neurotrophic trajectories observed between AD and HS astrocytes.

P043

LONG PENTRAXIN-3 AS A NEW THERAPEUTIC TARGET FOR MEDULLOBLASTOMA

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common malignant brain tumor in children, originating from distinct neural stem cell populations and classified into four subgroups: WNT, SHH, group 3, and group 4. Despite standard treatments, which often cause severe neurocognitive and long-term neurological side effects, 30–40% of 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, has unclear roles in cancer, acting as either an oncosuppressor or a pro-tumoral factor. Although substantial evidence supports the involvement of PTX3 in various cancers, no data is available about the impact of PTX3 in MB.

METHODS

Database, cell lines and patients' sample analyses confirmed the preferential expression of PTX3 in SHH-MB group. Then, PTX3 was knocked-down by short hairpin RNA (shRNA) in 3 different SHH-MB cell lines (DAOY, UW-228 and ONS-76) and knocked-out (KO), by CRISPR/Cas9, in DAOY cells line. Silenced cells and KO clones were used to elucidate the role of PTX3 in SHH-MB performing both in vitro and in vivo assays.

RESULTS

Our results show that PTX3 is expressed at low levels across the MB-groups and is overexpressed in the SHH group. In vitro assays revealed that both the silencing and the KO of PTX3 in DAOY cells significantly impaired key tumor features, including proliferation, migration and clonogenic potential.
In vivo, PTX3-KO clones exhibited a reduced tumor growth when implanted subcutaneously in immune-deficient mice, and IHC showed reduced proliferation (Ki67+) and vascularization (CD31+) in PTX3 KO tumors. The impaired angiogenic potential was further demonstrated in the chicken embryo chorioallantoic membrane (CAM) assay, and in vitro data revealed that PTX3 deficiency or downregulation was accompanied by reduced the production of pro-angiogenic factors and diminished angiogenic activation potential.
Mechanistically, we observed that PTX3 exerts its pro-tumor activity in SHH-MB by activating TLR4/IRAK1/GSK-3 β/β-catenin signaling in SHH-MB tumor cells.
Finally, under therapeutic perspective, targeting PTX3 directly may be a promising strategy, as its depletion increased the sensitivity of SHH-MB cells to radiotherapy and chemotherapy in vitro

CONCLUSIONS

Collectively, these data support the hypothesis of a pro-oncogenic effect of PTX3 in SHH-MB and uncover the importance of the PTX3/TLR4 axis for therapeutic and prognostic exploitation in SHH-MB

P044

MITOCHONDRIAL FISSION DRIVES AN ACTIONABLE METABOLIC DEPENDENCY IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Elevated mitochondrial fragmentation plays a crucial role in driving metabolic rewiring, contributing to tumor development. In this study, we investigated the functional significance of mitochondrial fission factor (MFF) and its partner Drp1—key drivers of mitochondrial fragmentation—in the pathobiology of multiple myeloma (MM).

METHODS

Mitochondrial morphology was analyzed by TEM, while cell viability was assessed using the CellTiter-Glo assay. Transcriptomic profiling was performed via Illumina NGS. mRNA and protein levels were measured by qRT-PCR and Western blot, respectively. In vivo anti-tumor efficacy was tested in MM-bearing NOD-SCID mice. Mitochondrial oxygen consumption (OCR) was evaluated using high-resolution respirometry.

RESULTS

Using a combination of in vitro and in vivo approaches, we demonstrated that blunting mitochondrial fission—via inhibition of MFF and Drp1 using shRNAs, siRNAs, LNA gapmeR antisense oligonucleotides, or novel selective Drp1 inhibitors—disrupts the bioenergetics of malignant plasma cells by inducing a metabolic shift from oxidative phosphorylation (OXPHOS) to glycolysis, thereby exerting anti-myeloma activity. Notably, the effects of MFF or Drp1 inhibition were enhanced by lactate supplementation or drugs that promote lactate accumulation, such as AZD3965 and Syrosingopine. These treatments increased the sensitivity of MM cells to mitochondrial fission inhibitors, underscoring a link between mitochondrial fission and lactate metabolism.
Furthermore, MFF and Drp1 were found to play a critical role in mediating resistance to bortezomib—a process driven in part by lactate—since combining lactate transporter inhibitors with bortezomib produced synergistic anti-tumor effects.

CONCLUSIONS

Targeting mitochondrial fission disrupts the metabolic adaptability of MM cells and enhances the effectiveness of lactate transporter inhibitors, overcoming resistance to standard-of-care-treatments like bortezomib.

P045

GEMCITABINE PROMOTES ASTROCYTE SENESCENCE: A COMPARATIVE STUDY BETWEEN ALZHEIMER'S DISEASE AND HEALTHY CELLS

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BACKGROUND-AIM

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline, amyloid-β (Aβ) plaques, and neurofibrillary tangles. A key early driver of AD is neuroinflammation, to which astrocytes, essential for CNS homeostasis, contribute when dysfunctional. Cellular senescence, a hallmark of aging, is implicated in age-related diseases. Senescent cells lose normal function and develop a pro-inflammatory secretory phenotype (SASP), leading to tissue degeneration and chronic low-grade inflammation (inflammaging). In AD, astrocytes accumulate in a senescent state, worsening neuroinflammation. Understanding how senescence differs in healthy and AD astrocytes is crucial. We used gemcitabine (GEM), a DNA-damaging agent capable of crossing the blood-brain barrier and used clinically in glioblastoma, to model senescence in vitro. This study investigates GEM-induced senescence in primary astrocytes from healthy donors and AD patients, and their differential susceptibility.

METHODS

Primary human astrocytes from healthy and AD donors were exposed to GEM (0.5, 1, 5, 10 μM) for 72 h to generate a dose-response curve. Based on cell viability (MTT assay), 1 μM was selected. Apoptosis was evaluated by Annexin V/PI. Senescence was assessed after 72 h GEM treatment followed by 72 h in fresh medium via SA-β-Gal staining, flow cytometry (cell cycle), Trypan Blue cell counting, and Western blot for p14ARF, pRB, Lamin B1, γH2AX, and macroH2A.1.

RESULTS

GEM at 1 μM effectively induced senescence with acceptable viability. Higher doses were excluded due to cytotoxicity. After 72 h, ~30% of cells (healthy and AD) were dead. Cell cycle analysis showed G1 arrest in treated cells, supported by stable cell counts versus proliferating controls. Senescence markers indicated stronger induction in AD astrocytes: SA-β-Gal activity was higher (~43%) compared to healthy (~19%), along with increased p14ARF and γH2AX. Lamin B1 and pRB were similarly reduced; macroH2A.1 was unchanged.

CONCLUSIONS

Low-dose GEM induces senescence in both healthy and AD astrocytes, with greater susceptibility in AD cells. This model offers a valuable tool to study astrocyte aging and AD mechanisms, and to screen senescence-targeting interventions.

P046

ISOLATION OF SYNTHETIC NANOBODIES AGAINST SERPINB3

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BACKGROUND-AIM

Squamous cell carcinoma antigen 1 (SCCA1) another name for SERPINB3 belongs to the serine protease inhibitor (serpin) family which is expressed by epithelial cells, leukocytes, and primary liver tumors. SERPINB3 mediates various processes including regulation of inflammatory response, metabolic, and cell death. Both autocrine and paracrine signaling pathways are impacted by SERPINB3s inhibition of cysteine proteases including papain and cathepsin K, L and S. Because of its role in the development of fibrosis cancer and cellular defense against oxidative stress it is important in both physiological and pathological contexts. Being linked to tumor growth and a poor prognosis makes SERPINB3 an attractive target for treatment.

METHODS

To isolate nanobodies (sybodies) against SERPINB3 this study used a hybrid selection strategy that combined one round of ribosome display with two subsequent rounds of phage display. Phage display enrichment and binder screening by ELISA were performed after the ribosome display step which used mRNA-encoded sybody (synthetic nanobody) libraries. Strategic optimization of the selection process was done to reduce the risk of non-specific enrichment and binder variability limitation. The FX (fragment exchange) cloning system transfered sybody-encoding sequences from the phagemid to expression vectors without PCR amplification which prevent unintentional rearrangement of complementarity-determining regions (CDRs). Quantitative PCR (qPCR) was used to track the enrichment efficiency at each selection round enabling real-time assessment and selection pipeline troubleshooting.

RESULTS

Following ribosome and phage display selections, ELISA screening confirmed that five sybody clones had more than 1.5-fold enrichment. Three were selected based on their binding strength and sequence diversity. These were successfully expressed in E.coli and then purified for additional biochemical and functional analysis.

CONCLUSIONS

These sybodies provide a strong foundation for SERPINB3 functional research and for developing therapeutic and diagnostic nanobody-based systems that target cancers associated with SERPINB3.

P047

AFFINITY MATURATION OF ANTIBODIES IN THE 2-PHENYL-5-OXAZOLONE SYSTEM

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BACKGROUND-AIM

Affinity maturation of antibodies is the process whereby more efficient antibodies are produced through somatic hypermutation and antigen-guided selection. No extensive study is yet available concerning the relationship between somatic mutations and their structural counterpart. The antibody response to 2-phenyl-5-oxazolone has been thoroughly investigated from the genetic point of view. It consists of three antibody classes, with each member of each class derived from a unique pair of VH and VL germline genes by somatic hypermutation. In this project, we are investigating the structure of the VH and VL domains of 10 representative antibodies.

METHODS

The VH and VL domains of each antibody are being expressed as recombinant scFvs or Fabs, crystallised, and their structure determined by X-ray crystallography.

RESULTS

The seven structures available, combined with models of the missing antibodies, allow an initial definition of the strategies adopted. In class I, maturation is bound to improvement of surface complementarity, especially at the top of the binding pocket, and in surface charge changes. In class II the maturation strategy seems to be based on the increase of the interacting surface, and on the introduction of a specific bond with the oxazolone ring. In class III, where the low and high affinity antibodies differ by 8 mutations, the increase in affinity is mainly determined by the improvement in the surface complementarity by removal of a bulky phenylalanine, which allows a better tightening of the two sides of the binding site. Interestingly, we have demonstrated by site-directed mutagenesis that a class I antibody is highly unstable in scFv, Fab and full-length antibody formats, raising the issue of the physiological role of such an antibody.

CONCLUSIONS

These results are relevant to determine the principles underlying affinity maturation of antibodies in both this and other antibody responses.

P048

THE METABOLIC BENEFITS OF CD300E: UNLOCKING THE IMMUNE RECEPTOR'S ROLE IN COMBATING OBESITY-RELATING DISORDERS

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BACKGROUND-AIM

A hallmark of obese adipose tissue is the accumulation of adipose tissue macrophages (ATMs), which cluster around hypertrophic adipocytes. These lipid-laden ATMs, also known as lipid-associated macrophages (LAMs), activate transcriptional programs involved in lipid uptake, lysosomal processing, and fatty acid metabolism. Recent evidence suggests that impaired lysosomal function in ATMs promotes lipid retention, triggering the release of anti-lipolytic factors that suppress adipocyte lipolysis — indicating a potential role for ATMs as sensors of local lipid overload.
In this study, we employed a KO mouse model to investigate the role of the immune receptor CD300e in modulating the adipocyte–macrophage crosstalk and its contribution to adipose tissue remodelling and systemic glucose homeostasis under obesogenic conditions.

METHODS

Wild-type (WT) and Cd300e$^{-/-}$ (KO) mice were fed a high-fat diet (HFD) for 16 weeks. Plasma, adipose tissue, and liver were collected for histological, lipidomic, and gene expression analyses. Glucose and insulin tolerance tests (GTT and ITT) were performed prior to sacrifice.

RESULTS

Loss of CD300e led to pronounced adipocyte hypertrophy in both visceral and subcutaneous fat depots. KO mice exhibited more severe hepatic steatosis compared to WT. Metabolically, KO mice showed worsened glucose intolerance and insulin resistance. At the cellular level, CD300e-deficient ATMs accumulated more lipids, while adipocytes from KO mice shifted toward enhanced lipogenesis and reduced lipolysis.

CONCLUSIONS

These findings advance our knowledge on immunometabolic crosstalk governing metabolic homeostasis within adipose tissue and identify CD300e as a critical sensor modulating lipid handling in ATMs, thus maintaining the metabolic dialogue between these cells and adipocytes. The absence of CD300e destabilizes adipose homeostasis, driving adiposopathy and systemic metabolic dysfunction. Therapeutically, targeting CD300e may represent a novel strategy to restore adipose tissue function and combat obesity-related metabolic complications.

P049

CARDIOMETABOLIC RISK IN OBESE WOMEN: THROMBOSPONDIN-1 AND ENDOTHELIN-1 AS NOVEL BIOMARKERS OF ADIPOCYTE DYSFUNCTION

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BACKGROUND-AIM

Thrombospondin-1 (TSP1) is a matricellular glycoprotein that orchestrates metabolic, thrombotic and inflammatory pathways. Although adipose-tissue TSP1 expression associates with endocrine dysfunction and insulin resistance, its link to obesity severity remains unclear. Endothelin-1 (ET1), a vasoactive peptide modulated by TSP1, may further amplify cardiometabolic risk in obese individuals. We tested whether adipose expansion and hypoxia modulate TSP1 release and whether circulating TSP1 and ET1 track adipocyte dysfunction across obesity grades.

METHODS

Differentiated 3T3-L1 adipocytes were exposed to hypoxia (2 % O_2, 24 h). Secretomes were analyzed by mass spectrometry and immunoblotting. TSP1 and hypoxia markers were quantified in visceral fat from C57BL/6 mice fed a high-fat diet. In parallel, 55 non-diabetic obese women (18-55 y; no cardiovascular history) were enrolled. Anthropometry, metabolic and inflammatory indices were collected; plasma TSP1/ET1 were measured by ELISA. Participants were stratified by body-mass index (BMI 30-34.9 kg/m²: class I; ≥35 kg/m²: class II/III).

RESULTS

Hypoxia reduced TSP1 secretion by cultured adipocytes. High-fat feeding induced adipose hypoxia and reproduced TSP1 down-regulation in vivo. In the clinical cohort (median BMI 33.7 kg m#²), 52 % were class II/III and 69.8 % displayed insulin resistance (HOMA-IR > 2.5) despite normal glucose tolerance. Circulating TSP1 tended to be lower in class II/III and correlated with HbA1c (r = 0.391), platelet count (r = 0.411) and leukocytes (r = 0.354). ET1 was likewise reduced in severe obesity and correlated positively with liver enzymes and triglycerides, but inversely with platelets and D-dimer. Platelet-normalized TSP1, reflecting adipocyte origin, was markedly reduced in class II/III, supporting hypoxia-driven secretory impairment in visceral fat.

CONCLUSIONS

Circulating TSP1, normalized to platelet count, mirrors the degree of adipose hypoxia and secretory failure in severe obesity. The decline of circulating TSP1 coupled with ET1, may identify an early stage of cardiometabolic vulnerability in obese women, who are otherwise protected from cardiovascular events until menopause, potentially representing non-invasive biomarkers for cardiometabolic risk stratification.

P050

ATM MUTATIONS IN THE HBOC SYNDROME, AN EMERGING PERSPECTIVE

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BACKGROUND-AIM

Hereditary Breast and Ovarian Cancer (HBOC) syndrome, initially defined by BRCA1/2 mutations, now includes other susceptibility genes such as ATM. While biallelic ATM mutations cause ataxia-telangiectasia (a rare recessive neurodegenerative disorder), heterozygous pathogenic variants (PVs) have been associated to HBOC. The aim of our study was to evaluate the prevalence of the variants in the ATM gene, including PVs, Variants of Uncertain Significance (VUS) and novel variants, in HBOC patients and to explore the genotype-phenotype correlation. Bioinformatic analysis was performed to assess the clinical impact of VUS and novel variants and co-segregation analysis was carried out when feasible.

METHODS

250 patients, fulfilling HBOC diagnostic criteria, underwent counseling and testing through NGS for HBOC-associated genes. Variants were classified using ClinVar and LOVD databases. VUS and novel variants underwent bioinformatic analysis to predict their potential clinical impact and were subsequently classified into five risk categories based on the in silico results. We also evaluated the clinical features of patients carrying PVs and their family members and, when feasible, genetic testing was extended to relatives to perform co-segregation analysis.

RESULTS

In our cohort, ATM was the most frequently mutated gene after BRCA1 and BRCA2. 2.8% (7/250) of patients harbored a PV in ATM while 7.2% (18/250) carried a VUS, and 2% (5/250) had a novel variant. Among the 12 identified VUS, bioinformatic analysis classified 1 as benign (class V), 7 as likely benign (class IV), and 4 as 'of uncertain significance' (class III). All 5 novel variants resulted benign (class V). Analysis of the clinical features of patients carrying a PV in ATM and their family members revealed that breast cancer was the most frequent malignancy (54%), followed by ovarian cancer (11%) and melanoma (11%), CRC (7%), lung cancer (7%), prostate cancer (7%), gastric cancer (4%).

CONCLUSIONS

Our findings indicate that a significant subset (2.8%) of patients with HBOC syndrome carry PVs in the ATM gene. In silico analysis showed that most VUS (67%) were benign or likely benign hence not implicated in the syndrome. Clinical phenotype evaluation revealed the recurrence of tumors typically associated with BRCA1/2 PVs. Expanding genetic testing beyond BRCA is key to improving hereditary cancer diagnosis and guiding precision oncology.

P051

MAMMATYPER®: MOLECULAR PREDICTOR OF RESPONSE TO NEOADJUVANT THERAPY IN HER2-POSITIVE BREAST CANCER.

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BACKGROUND-AIM

Neoadjuvant therapy (NAT) has become the standard of care for most HER2-positive early breast cancer (BC). However, 25 to 50% of patients fail to achieve a pathological complete response (pCR). The study aims to assess the clinical utility of the CE/IVD MammaTyper® kit (Cerca Biotech) as a predictor of response in this setting.

METHODS

One-hundred and sixty-one HER2-positive/3+ IHC-score invasive BC patients were enrolled. All patients underwent trastuzumab-based NAT combined with a taxane backbone. Ethical approval was obtained. Three patients were excluded due to insufficient RNA amount, along with 4 patients treated with dual anti-HER2 blockade. Thus, 154 FFPE preoperatory core-biopsies were tested: 79 were hormone-positive (HR+) and 75 hormone-negative (HR-). Ninety-one subjects achieved a pCR while 63 attained a pathological partial response (pPR). MammaTyper®, a molecular in vitro diagnostic RT-qPCR test, was used to assess the relative mRNA expression levels of the ERBB2, ESR1, PGR and MKI67 genes. A machine-learning, Python-based Decision Tree Algorithm was used to predict pCR from the $\Delta\Delta$Cq values of the four genes alongside tumor size and nodal status (MTClin). Samples were stratified by MammaTyper® hormone receptor (ER and/or PgR) status. To balance interpretability and generalizability, key hyperparameters were tuned and GridSearchCV with a 5-fold cross-validation was used. Analytical accuracy was evaluated in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Funding: PNRR - M4.C2 - Tuscany Health Ecosystem - Spoke n.6, CUP I53C22000780001 (C.S., A.G.N.); PNRR - M4C2-I1.3 Project PE00000019 HEAL ITALIA, CUP E63C22002080006 (F.M., P.V.).

RESULTS

Two Decision Trees were selected showing high specificity and sensitivity, and plausible biomarker hierarchy. In detail, the tree for HR+ tumors showed 90.2% sensitivity, 86.8% specificity, 88.1% PPV, and 89.2% NPV. The decision tree for HR- tumors had a sensitivity of 96%, a specificity of 88%, a PPV of 94.1% and a NPV of 91.7%.

CONCLUSIONS

MTClin may discriminate patients with HER2-positive BC who will achieve pCR from those who will not, representing a powerful decision tool in terms of escalation/de-escalation of treatment approaches.

P052

IN SILICO AND IN VITRO EVALUATION OF BONE MARROW CD8+ TISSUE-RESIDENT MEMORY CELLS IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Multiple Myeloma (MM) is a complex blood cancer caused by the uncontrolled proliferation of abnormal plasma cells in the bone marrow (BM). It progresses through a multi-step transformation from monoclonal gammopathy of undetermined significance (MGUS) to smoldering myeloma (SMM) before developing into MM.

Recent research highlights the role of $CD8^+$ T memory cells in MM progression, particularly the decline of $CD8^+$ T memory stem cells in MGUS, which may trigger disease advancement. This study focuses on $CD8^+$ tissue-resident memory T cells (TRM) and their significance in MM progression and immune defense mechanisms.

METHODS

By analyzing single-cell RNA sequence data on over 600,000 cells from the BM of 183 patients across various disease stages, the study emphasizes the molecular and phenotypic evolution of $CD8^+$ TRM cells. Further analysis of 161,000 high-quality $CD8^+$ T cells and immunophenotyping by flow cytometry of a 35-patient independent cohort reveals a correlation between $CD8^+$ TRM cell prevalence and patient outcomes.

RESULTS

In MM, $CD8^+$ TRM cells displays diminished cytotoxic capabilities and increased expression of exhaustion markers. Moreover, the abundance of effector memory and terminally differentiated $CD8^+$ TRM cells are inversely correlated with patient progression-free survival, indicating its potential as a prognostic marker and a target for therapeutic intervention.

To further investigate $CD8^+$ TRM functionality, we expanded these cells from MM patients' BM. Upon stimulation, $CD8^+$ TRM cells developed a cytokine response upon recognition of autologous myeloma cells and this response was inhibited by an anti-CD3 monoclonal antibody.

CONCLUSIONS

In conclusion, $CD8^+$ TRM cells increase during MM progression but often exhibit functional exhaustion or suppression in advanced stages (MM and RRMM) and their frequency correlate with poor PFS. Nevertheless, the less differentiated $CD8^+$ TRM subsets show a proliferative potential and retain TCR-dependent cytotoxicity against MM cells, supporting their potential for immunotherapy.

P053

CLINICALLY-ORIENTED AND PATIENT-ADAPTED REPORTING OF BREAST CANCER GRADE PREDICTIONS USING LARGE LANGUAGE MODELS (LLMS)

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BACKGROUND-AIM

Histological tumor grade remains a key breast cancer (BC) prognostic indicator, with well-established correlations to patient outcomes. However, its assessment is subject to interobserver variability, potentially leading to inconsistent classification of tumor aggressiveness. To overcome this limitation, transcriptomic signatures predictive of histological grade offer a promising and objective tool to support diagnostic accuracy and clinical decision-making. Furthermore, advances in large language models (LLMs) provide a new opportunity to translate complex gene-level insights into human-friendly, insightful reports, enhancing the interpretability and utility of molecular diagnostics in clinical practice.

METHODS

The methodology workflow comprises three main steps: (i) ensemble model training for BC grade prediction and gene selection; (ii) validation of the prognostic relevance of the selected genes via survival analysis; (iii) explainable BC grade prediction coupled with LLM-driven generation of personalized reports.

RESULTS

By applying machine learning (ML) techniques to RNA-sequencing data, we identified a gene expression signature predictive of BC grade in a large cohort of patients. Moreover, the survival analyses further corroborated the prognostic role of the identified genes. Thereafter, we leveraged LLMs to produce natural language reports for clinicians and patients. Our approach enabled both accurate grade classification and interpretable stakeholder-specific reports that meet the distinct informational needs of clinicians and patients.

CONCLUSIONS

We identified a gene signature predictive of BC grade, enabling a unified and interpretable pipeline for grade classification and validation. By integrating explainable artificial intelligence with LLM-driven reporting, we deliver clinically relevant outputs in clear, stakeholderspecific language. This approach facilitates actionable insights for clinicians and empowers patients with personalized information, supporting more informed and patient-centered decision-making in oncology care.

P054

ROLE OF ANTI-MDA5 ANTIBODIES IN THE PATHOGENESIS OF DERMATOMYOSITIS AND RELATED RP-ILD

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BACKGROUND-AIM

MDA5, encoded by the IFIH1 gene, is a cytosolic receptor of the RIG-I-like family that detects long double-stranded viral RNA. Upon activation, MDA5 engages MAVS to initiate an inflammatory cascade involving IRF3, IRF7, and NF-κB, culminating in type I interferon production. Anti-MDA5 antibodies are markers of a dermatomyositis subset (anti-MDA5 syndrome) characterized by rapidly progressive interstitial lung disease (RP-ILD), which has poor prognosis and systemic involvement. Although diagnostic, the role of these antibodies in pathogenesis remains unclear.
To investigate this, we pursued two complementary approaches: (1) isolation of polyclonal anti-MDA5 antibodies from patient serum, and (2) identification of single antibody specificities via single-cell techniques.

METHODS

Patient-derived antibodies were purified by affinity chromatography, isotyped, and applied to A549 cells—used as a model of alveolar Type II pneumocytes—for viability assays (RTCA and MTT). To study monoclonal antibody features, PBMCs were collected and sorted for CD19 and MDA5 expression. Due to the rarity of CD19+ MDA5+ cells (~400 cells in 3×10#), NGS was unfeasible; instead, single cells were seeded into 96-well plates (BD FACS Aria II), followed by retrotranscription and PCR to identify VH and VL gene pairs.

RESULTS

Polyclonal antibodies, found to be IgG1 with mostly kappa chains, reduced A549 cell viability by 17% at both 1 µg/ml and 5 µg/ml and by 19 and 28%, respectively, when combined with equimolar MDA5. RTCA showed a 25% decrease in impedance after 80 h with antibodies alone and ~50% with both antibodies and MDA5.
Single-cell analysis revealed recurrent VH (e.g., IGHV3-72) and VL sequences shared with anti-viral antibodies, including those targeting HIV and SARS-CoV. Further analysis is ongoing to clone these antibodies, define epitopes, and evaluate affinity maturation.

CONCLUSIONS

The reduction in A549 viability suggests a direct role for anti-MDA5 antibodies, potentially through immune complex effects. Shared VH/VL genes with antiviral antibodies support a viral link in disease pathogenesis. Further steps are ongoing to clarify the pathogenic role of anti-MDA5 antibodies in this rare myositis subset.

P055

THE LOSS OF DDB2-PCNA INTERACTION INFLUENCES CELL GROW AND GENOME INSTABILITY IN UV-IRRADIATED HUMAN CELLS

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BACKGROUND-AIM

The DDB2 protein plays a key role in the early steps of Global Genome Nucleotide Excision Repair (GG-NER). Our previous studies have demonstrated that the interaction between DDB2 and PCNA proteins is essential for the efficient removal of UV-C-induced DNA lesions. Cells expressing a mutant DDB2 unable to bind PCNA ($DDB2^{PCNA-}$) exhibit delayed NER and tumor-like behavior.

METHODS

Soft agar clonogenic assays were performed using HEK293 cells, untransfected or stably expressing either wild-type or mutant DDB2, both untreated and UV-C irradiated (10 J/m²). Only the cells expressing $DDB2^{PCNA-}$ were able to grow, and two resistant clones (called Clone 1 and Clone 2), were isolated and expanded. Protein expression was assessed by flow cytometry, microscopy, and Western blot (WB). Cell adhesion was analyzed using iCELLigence biosensor technology and cell migration was evaluated through Boyden chamber and wound healing assays.

RESULTS

Clone analysis aimed to explore tumor-like phenotypes in comparison to the parental cell line. In proliferation assays, Clone 2 showed a high colony-forming ability even at the highest cisplatin concentration, whereas the parental line was more resistant to HU treatment. Both clones exhibited reduced adhesion, faster wound closing, and increased levels of CD117, CD44, and OCT4, markers linked to cancer progression and cellular plasticity. WB analysis revealed different expression of protein involved in EMT process (SNAI1, Vimentin and ZEB1) and transcription factor, such as NF-kB, compared to the parental line. The PKM protein, involved in metabolism processes, was also examined. Zymography was used to assess matrix metalloproteinases 2 and 9 activities. Given the importance of the tumor microenvironment, especially macrophages, we tested the effect of conditioned media from different cell cultures on THP-1 monocytes. Preliminary results suggest monocyte differentiation and increased CD80 expression. Additionally, both clones were able to form 3D spheroids on agar faster than the parental line.

CONCLUSIONS

These findings confirm that $DDB2^{PCNA-}$ cells exhibit tumor-like traits and highlight significant differences between the Clones and the parental cells. Their observed heterogeneity requires further investigation to better understand the molecular mechanisms involved.

P056

CIRCADIAN RHYTHM IN INFECTIONS: NEW INSIGHTS FOR DIAGNOSIS AND THERAPY

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BACKGROUND-AIM

Organisms across all domains of life have adapted to circadian changes of the environment and regulate their behavior and physiology accordingly. During host-pathogen interactions, daily fluctuations in the availability of nutrients drive a time-dependent host immune response, strongly influencing microbial colonization, level of inflammation and damage to the host, and altering the equilibrium between commensal and invading microorganisms. Among nutrients, the amino acid tryptophan and its downstream metabolites are crucial regulators of both immunity and the circadian clock. A circadian regulation of the kynurenine pathway in the host response against Aspergillus fumigatus opportunistic infection has been recently demonstrated by our studies.

METHODS

In this study, we performed fungal intragastric infection with Candida albicans at different time of the day-night cycle, and in mice genetically deficient for main components of the tryptophan metabolic pathway. In these mice, we assessed degree of colonization, level of inflammation and tissue damage. Moreover, a comprehensive analysis of immune and metabolic profile was performed looking for potential circadian biomarkers of infection.

RESULTS

We demostrated that a time-dependent host immune response occurs during gastrointestinal opportunistic infection with Candida albicans. Such diurnal regulation is driven by the circadian production of metabolites modulating the balance between resistance of the host and virulence of the pathogen, leading to a time-dependent prevalence of inflammation or tolerance.

CONCLUSIONS

Our results demonstrate that the circadian rhythm at the intersection between metabolism and immune response underlies diurnal changes in host–pathogen interaction, thus paving the way for circadian-based interventions for diagnosis and antimicrobial therapy in opportunistic infections.

P057

A GUT-KIDNEY MULTI-ORGAN-ON-A-CHIP AS A MICROPHYSIOLOGICAL MODEL FOR ENTERIC HYPEROXALURIA: FROM MICROFLUIDIC MULTI-ORGAN SYSTEMS PATHOGENESIS TO A MICROBIAL METABOLITE-BASED THERAPEUTIC STRATEGY

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BACKGROUND-AIM

Hyperoxaluria, a medical condition characterized by calcium oxalate urinary super saturation, determines decreased renal function and in severe cases end-stage renal failure. Hyperoxaluria may be caused by genetic defects in the hepatic metabolism of glyoxylate, the direct precursor of oxalate (Primary Hyperoxaluria, PH), or be secondary to conditions that lead to increased absorption of oxalate (Secondary Hyperoxaluria, SH), among which pathologies characterized by intestinal malabsorption, such as inflammatory bowel disease (IBD), represent a specific type (Enteric hyperoxaluria, EH). While the understanding of the pathogenesis of PH has considerably moved forward in recent years, few studies have explored the causal mechanisms of EH hampering diagnostic and therapeutic advances. The possibility to model EH in a microphysiological system would allow us to better understand disease pathogenesis and pave the way for novel therapeutic strategies. Organ-on-a-chip (OoC) represents an innovative approach to study physiopathological processes, providing a more physiological platform for disease modeling and drug discovery over conventional in vitro and in vivo models.

METHODS

In this work, we attempted to model EH 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 [3]. Initially, we set-up a kidney model 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 compartments, respectively. To mimic the intestinal barrierCaco2 cells were cultured in a transwell system.

RESULTS

Oxalate administration in the apical compartment of Caco2 cells to mimic dietary intake resulted in absorption and excretion in the urine, an effect that was enhanced by treatment with Dextran Sodium Sulfate (DSS), a chemical commonly used to model inflammatory bowel disease. Oxalate exposure to renal cells resulted in increased inflammatory response, an effect that was reduced by microbial metabolites, in addition to their role in protecting the intestinal barrier.

CONCLUSIONS

These results support the role of microbial metabolites in mitigating EH by performing both local and distant effects along the gut-kidney pathogenic axis, thus paving the way for their therapeutic development.

P058

IMMUNOTHERAPY OF INFECTIONS: THE CASE OF AF-CAR NK CELLS AGAINST ASPERGILLUS FUMIGATUS

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BACKGROUND-AIM

Invasive pulmonary aspergillosis (IPA) poses an escalating threat to immunocompromised patients, driven by increasing risk factors and growing resistance to existing antifungal treatments. To address this challenge, over the past decade, there have been pivotal developments in adoptive T cell therapy to produce and expand clinically relevant antigen-specific T cell products. Their use in clinical conditions, especially in treating cancer as well as for prevention of life-threatening viral infections in allogeneic transplant recipients, demonstrated safety and clinical efficacy, providing a potential therapeutic option for patients who are unresponsive to standard treatments. We recently developed a Chimeric Antigen Receptor targeting Aspergillus fumigatus (Af-CAR). Af-CAR successfully redirected the specificity of T cells toward A. fumigatus, inducing potent anti-fungal responses, particularly in CD8+ T cells.

METHODS

In an experimental murine model of Aspergillus fumigatus infection, we have tested the use of CAR NK cells as an attractive new therapeutic alternative, leveraging their intrinsic antifungal activity and low risk of cytokine release syndrome. Mice were evaluated for survival, fungal burden, cell recruitment in the bronchoalveolar lavage fluid and lung histology.

RESULTS

In a murine IPA model, Af-CAR NK cells treatment recruited mononuclear cells while reducing neutrophil infiltration, thus lowering fungal burden and alleviating lung tissue damage. Notably, adoptive transfer of Af-CAR NK cells improved survival in neutropenic IPA mouse models.

CONCLUSIONS

In conclusion, the Af-CAR induces a distinct phenotype in NK cells which enhances their direct antifungal activity and promotes a protective immune response. These findings highlight the potential of Af-CAR NK cells as a promising therapeutic approach for IPA.

P059

EXPLORING THE MOLECULAR SIGNATURE OF TRIPLE NEGATIVE BREAST CANCER USING COMPUTATIONAL AND TRANSCRIPTOMIC STRATEGIES.

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BACKGROUND-AIM

Triple negative breast cancer (TNBC) accounts roughly for 15-20% of breast cancer cases and is the most hard-to treat subtype since it cannot take advantage from hormonal or target therapy. In this view, the identification of novel biomarkers and potential therapeutic targets is urgently needed. The aim of our project is to contribute to unravel the molecular profile of TNBC patients by in silico and RNASeq approaches.

METHODS

In this study, we performed an in-silico analysis on publicly available TCGA datasets to identify differences in the transcriptomic profiles between TNBC and other breast cancer subtypes; moreover, RNAseq was applied in a cohort of 12 FFPE TNBC samples in order to define their molecular landscape. A thorough bioinformatic analysis was applied in both settings.

RESULTS

In silico analysis showed that the basal subtype was enriched in IL-17 signalling pathway and in genes involved in ion channel complexes and extracellular matrix. Compared to healthy controls, TNBC samples displayed an upregulation of genes associated with protein targeting to the membrane and to the endoplasmic reticulum, and downregulation in collagen-containing extracellular matrix, ribosome components and protein binding. RNASeq also identified novel lncRNAs. Enrichment analysis of co-expressed genes highlighted pathways involved in various subtypes of breast cancer (Basal-like, familial breast cancer, HER2-negative and invasive ductal breast carcinoma), all tightly interconnected. KEGG pathway analysis showed enrichment in cytokine-cytokine receptor interactions, various types of chemical carcinogenesis (receptor activation, DNA adducts), basal cell carcinoma and miRNAs in cancer.

CONCLUSIONS

TNBC still represent a big challenge for clinicians thus unravelling the molecular landscape will pave the way for more efficacious targeted therapies. In this scenario, a promising class of novel targets is represented by ion channels and complexes that can be easily tuned and targeted for both diagnostic and therapeutic purposes.

P060

IN VITRO EFFECTS OF STILBENES COMBINATION WITH CISPLATIN IN NON-SMALL CELL LUNG CANCER TREATMENT

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BACKGROUND-AIM

Lung cancer is one of the most relevant tumours in the world. Its eradication remains an elusive clinical goal, primarily due to drug resistance and toxicity associated with chemotherapeutic agents, such as cisplatin (CDDP). Resveratrol (RSV) is a stilbene-based compound that has been widely investigated due to its multiple biological properties, including anti-proliferative and anti-oxidant effects. Its analogue, 4,4'-dihydroxy-trans-stilbene (DHS), has shown superior activities, both in vitro and in vivo studies.
This work explores the effect of stilbenes combination with cisplatin, focusing on their effects in non-small cell lung cancer (NSCLC) cell lines.

METHODS

NSCLC cell lines (A549, Calu1 and SKMES1) were co-treated with stilbenes (30μM) and CDDP (5-10-15-20-25μM) for 72h to evaluate their proliferation by IncuCyte. Afterwards, to evaluate acute effect, other experiments were performed only on A549 using stilbenes 30μM and CDDP 25μM for 24h. Cytotoxicity was evaluated by the MTT assay; DNA damage was analysed by the phosphorylation of histone H2AX; cell cycle distribution was assessed by flow cytometry after propidium iodide staining.

RESULTS

The IncuCyte proliferation was highly reduced after co-treatments, compared to controls, in particular with DHS and CDDP in all the cell lines. Furthermore, in A549 was observed an increased cytotoxicity and DNA damage, compared to single treatments and controls. Still, alteration in cell cycle progression was relevant: complementary effect on DNA gathering with the combinations of stilbenes and CDDP was noted, especially for DHS treatment with CDDP, with a specific accumulation of cells in the sub-G0/G1 phases.

CONCLUSIONS

These findings suggest synergistic effects of CDDP and stilbenes, particularly DHS, on A549 cells, through enhanced DNA damage and imbalance in cell cycle distribution, proposing a novel strategy to overcome resistance mechanisms.

P061

IMPACT OF ASPARAGINASE ON TRIPLE NEGATIVE BREAST CANCER CELL LINES

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BACKGROUND-AIM

Asparaginase (ASNase), a first-line drug for Acute Lymphoblastic Leukemia, exerts its effect by hydrolyzing asparagine (Asn) and glutamine (Gln), two conditionally essential amino acids for leukemic cells. We tested it on two triple negative breast cancer (TNBC) cell lines, BT549 and MDAMB231, considering the limited treatment options for this cancer.

METHODS

We evaluated the effect of increasing ASNase concentrations on short- (72 h, growth curve) and long-term (10 d, clonogenic assay) proliferation. Data were supported by real-time cell analysis (RTCA, iCELLigence). Cell cycle was analyzed marking newly synthesized DNA with 5-ethynyl-2'-deoxyuridine (EdU). Western blot was performed on markers of drug sensitivity (ASN-synthetase, ASNS, and GLN-synthetase, GS) and drug response (Akt, ERK and pS6).

RESULTS

In both cell lines, proliferation was significantly inhibited both in the short term (doubling time, CTR vs. 1 U/ml ASNase, BT549 18.3#2.7h vs. 54.8#7.5h, and MDAMB231 21.4#3.4h vs 37.5#5.1h), and in the long term (IC50, BT549 0.044±0.003 U/ml, MDAMB231 0.30±0.20 U/ml). Cell adhesion (measured by RTCA) of BT549 decreased significantly at 0.1, 0.5 and 1 U/ml.

Cell cycle analysis showed a canonical response (i.e., G1-blockade) in BT549 at 1 U/ml and an enrichment of cells, negative for newly synthesized DNA (EdU–) in the S-phase, at 3 U/ml, which means a less efficient DNA synthesis. Western blot on BT549 showed a significant increase in GS and ASNS expression at 0.5 and 1 U/ml, as a compensatory response mechanism to the amino acids' depletion, and a significant decrease of p53 at 3 U/ml due to a decrease in protein synthesis, highlighted by a significant decrease in S6 phosphorylation at the same dose. Interestingly, protein synthesis inhibition was accompanied by a non-significant increasing trend of ERK activation and absent reduction of the upstream regulator Akt.

CONCLUSIONS

Here we describe for the first time cell cycle, proliferation, adhesion and DNA synthesis inhibition in BT549, and, to a lower extent, MDAMB231 TNBC cell lines induced by amino acid depletion. Moreover, growth inhibition is independent from the Akt/mTORC1 canonical pathway. Further experiments are being carried out on TNBC spheroids to investigate ASNase mechanism and effects also in a 3D cancer model.

P062

IMPACT OF CONDITIONED MEDIUM FROM DDB2PCNA- EXPRESSING HEK293 CELLS ON MACROPHAGE ACTIVITY

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BACKGROUND-AIM

DNA damage-binding protein 2 (DDB2) plays a pivotal role in the initial recognition of DNA lesions induced by UV-C. It interacts directly with PCNA through a conserved PIP-box motif. Mutation in this PIP-box ($DDB2^{PCNA-}$), leading to defective DDB2 degradation and abnormal accumulation in cells, drives to reduced NER efficiency, persistent DNA damage, and a more aggressive cellular phenotype.

This study explores how expression of the mutant $^{DDB2PCNA-}$ protein affects macrophage functions "in vitro", potentially revealing a link between impaired DNA repair and inflammation related-tumour microenvironment remodelling.

METHODS

THP-1 monocytes were cultured in conditioned medium from HEK293 $DDB2^{Wt}$, or $DDB2^{PCNA-}$ cells, collected before and after UV-C (10 J/m²). Subsequently, co-culturing was performed with µ-Slide 2 Well using THP-1 as recipient cell and irradiated HEK293 $DDB2^{Wt}$ and $DDB2^{PCNA-}$ as feeder cells. The THP-1 cell morphology was evaluated using gentian violet staining, while immunofluorescence analysis was performed to examine the expression of CD80. Additionally, Western blot analysis was conducted on all irradiated cell lines to determine inflammatory protein levels.

RESULTS

The most significant changes occurred in cells exposed to $DDB2^{PCNA-}$ conditioned-medium (7d post-UV). Microscopic observations revealed that THP-1 cells exhibit pronounced macrophage-like morphology characterized by multiple cellular “pseudopodia”, whereas cells exposed to control or $DDB2^{Wt}$ medium show minimal morphological changes. Similar results were obtained in co-cultures experiments. Immunofluorescence studies demonstrated increased CD80 membrane localization in THP-1 cells exposed to $DDB2^{PCNA-}$ conditioned medium. Preliminary data from Western blot analysis, showed elevated IL-6, IL-8, and CD38 levels only in UV-treated HEK293 cells stably expressing the $DDB2^{PCNA-}$ mutant protein.

CONCLUSIONS

These findings support a potential link between the loss of DDB2-PCNA interaction and macrophage-driven inflammation, relevant for understanding tumour microenvironmental dynamics.

P063

ASSESSMENT OF THE LIAISON® S. PNEUMONIAE AG TEST FOR RAPID DIAGNOSIS OF PNEUMOCOCCAL INFECTIONS IN URINE SAMPLES

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BACKGROUND-AIM

Streptococcus pneumoniae is a major cause of community-acquired pneumonia and invasive infections, particularly in older adults and immunocompromised individuals. Rapid and accurate diagnosis is essential to guide appropriate therapy, improve patient outcomes, and reduce unnecessary antibiotic use. Urinary antigen detection provides a fast, non-invasive alternative to culture or PCR, especially when respiratory samples are unavailable or compromised by prior antibiotic treatment.

METHODS

This study evaluated the performance of the LIAISON® S. pneumoniae antigen assay, an automated chemiluminescent immunoassay (CLIA) designed for the qualitative detection of S. pneumoniae antigen in human urine. Analytical validation was conducted on 370 anonymized residual urine samples, including cases with suspected S. pneumoniae pneumonia and potential cross-reactivity with other pathogens.

RESULTS

Results were compared to the laboratory reference method, and discordant cases were further investigated using an alternative method (SD-Biosensor). The assay correctly identified 111 positive and 242 negative samples, with only a minimal number of equivocal or discrepant results. It demonstrated a sensitivity of 98.2% (95% CI: 93.8%–99.5%) and a specificity of 98.8% (95% CI: 96.5%–99.6%), confirming high diagnostic accuracy.

CONCLUSIONS

These findings support the clinical utility of the S. pneumoniae antigen assay as a reliable and rapid tool for the detection of pneumococcal infections using urine specimens. The high sensitivity and specificity, combined with the advantages of automation and ease of use, make this assay particularly valuable in routine diagnostic workflows. The routine implementation of the assay could contribute to optimizing the clinical management of suspected cases of pneumococcal disease.

P064

ASSESSMENT OF THE ANALYTICAL PERFORMANCE OF WIDAL-WRIGHT AND WEIL-FELIX REACTIONS ON THE AUTO-DAT INSTRUMENT

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BACKGROUND-AIM

Serological agglutination tests remain essential tools for detecting pathogen-specific antibodies, particularly for Salmonella, Brucella, and Rickettsia infections. To enhance standardization and reduce operator-dependent variability, DIESSE Diagnostica Senese has developed AUTO-DAT, a semi-automated system for reading and interpreting agglutination reactions.

METHODS

An analytical verification study was conducted at the Institute of Pathology, ASUFC, between February 2024 and March 2025, to evaluate the performance of the Widal-Wright (WW) and Weil-Felix (WF) tests on AUTO-DAT. The analysis focused on sensitivity, specificity, matrix effects (serum vs plasma), and comparison with a manual reference method (LTA).

RESULTS

A total of 446 anonymized residual clinical samples were analyzed, including spiked samples at various antibody titers and commercial positive controls. Specifically, 113 samples were used for parameter optimization (53 WW, 60 WF), 282 for external performance validation (188 WW, 94 WF), 31 for serum/plasma comparison, and 20 for cross-reactivity testing with unrelated antibodies.
AUTO-DAT achieved 100% sensitivity and specificity for both tests, with complete agreement with the reference method (Cohen's Kappa = 1). No matrix-related effects or significant cross-reactivity were observed.

CONCLUSIONS

These results confirm the suitability of AUTO-DAT for routine implementation in clinical laboratories, offering high analytical performance and compliance with regulatory requirements for semi-quantitative serological diagnostics.

P065

MOLECULAR CHARACTERIZATION OF CIRCULATING TUMOR CELLS IN METASTATIC BREAST CANCER USING SHALLOW WHOLE GENOME SEQUENCING (SWGS)

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BACKGROUND-AIM

Metastatic breast cancer (MBC) remains the leading cause of cancer-related death in women, largely due to difficulties in early detection of metastases and limited efficacy of current therapies. Circulating Tumor Cells (CTC), key players in metastatic progression, represent a minimally invasive biomarker for real-time tumor monitoring. Beyond enumeration, their molecular profiling may offer deeper prognostic insights and inform treatment decisions.

METHODS

We performed shallow Whole Genome Sequencing (sWGS) on 141 single CTC isolated from 10 patients with metastatic luminal breast cancer using DEPArray technology. After Whole Genome Amplification, sWGS was conducted on an Illumina MiSeq platform. Copy number alterations (CNA) were identified through a custom bioinformatic pipeline. The clinical relevance of recurrent CNA patterns found was evaluated in 466 breast cancer samples from The Cancer Genome Atlas (TCGA) using Kaplan-Meier survival analysis and log-rank testing.

RESULTS

6 recurrent CNA signatures were identified among CTC. 4 showed significant association with poor prognosis: 1p chromosome deletion, 1q amplification, X chromosome hemizygous deletion, and chromosome 4 deletion. These signatures also demonstrated prognostic relevance in primary breast cancer tissues from the TCGA dataset.

CONCLUSIONS

This study demonstrates the utility of sWGS for single-cell genomic profiling of CTC and tissues in breast cancer. The identification of CNA signatures associated with poor outcomes highlights their potential as prognostic biomarkers. The concordance with TCGA primary tumors supports their integration into personalized therapeutic strategies to improve clinical management.

P066

"TARGETED APOPTOSIS AND TUMOR GROWTH INHIBITION VIA A TRI-SPECIFIC ANTIBODY DIRECTED AGAINST HERG1/β1 INTEGRIN AND TRAIL RECEPTORS"

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BACKGROUND-AIM

In recent years, targeted therapies and immunotherapies have significantly advanced cancer treatment. One of the most promising strategies is the induction of tumor cell apoptosis via Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL), which binds to death receptors DR4 and DR5 on the plasma membrane of target cells. However, the clinical use of soluble TRAIL is limited by its short in vivo half-life and poor activity on DR5-expressing cells. Previous studies have shown that fusing TRAIL sequences with antibody-based moieties can overcome these limitations and enhance antitumor efficacy.
The aim of this study is to develop and characterize a novel tri-specific single-chain antibody that simultaneously targets the hERG1/β1 integrin complex and TRAIL receptors, in order to improve the selectivity and pro-apoptotic efficacy of TRAIL-based cancer therapy.

METHODS

We developed a novel therapeutic construct, scDb-hERG1-β1-TRAIL, consisting of a single-chain diabody (scDb) targeting the cancer-specific hERG1/β1 integrin complex, fused with three TRAIL sequences. This fusion combines the tumor-targeting capability and signaling modulation of the scDb with the pro-apoptotic activity of TRAIL. Antitumor activity was assessed in vitro, on breast cancer (BCa) cell lines, and using a novel microfluidic platform and in vivo in a mouse model of Triple-Negative Breast Cancer (TNBCa).

RESULTS

Treatment with scDb-hERG1-β1-TRAIL significantly reduced tumor cell proliferation and induced apoptotic cell death in BCa cells in vitro. In vivo, the antibody demonstrated marked antitumor efficacy in TNBCa-bearing mice, with a favorable pharmacokinetic and toxicity profile. The therapeutic effects were correlated with the expression of both the hERG1/β1 complex and TRAIL receptors on tumor cells.

CONCLUSIONS

The scDb-hERG1-β1-TRAIL antibody represents a promising therapeutic strategy for the treatment of hard-to-treat tumors, such as Triple-Negative Breast Cancer, by combining selective tumor targeting with TRAIL-induced apoptosis. Preclinical results support further development toward clinical application.

P067

ADDITIVE TOXIC EFFECTS OF PERSISTENT ORGANIC POLLUTANTS (POPS) MIXTURES ON PANCREATIC BETA CELLS

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BACKGROUND-AIM

Persistent organic pollutants (POPs) are chemicals that are particularly resistant to degradation, accumulate in living organisms and can exert toxic effects on both humans and wildlife. In particular, convincing evidence has been provided for a link of type 2 diabetes with POPs exposure. Recently, a new concern has been raised regarding the negative effects of low dose POPs like the current exposure levels. In the past, most studies have been performed using a one-chemical-at-time approach to study the adverse effects of the exposure to a single pollutant. However, humans are rarely exposed to single toxicants, but rather to complex mixture that can combine their effects. The aim of our research has been to evaluate the eventual additive toxic effects of a mixture containing different POPs on pancreatic beta cells.

METHODS

INS-1 832/13 cells, a genetically modified INS-1 subclone selected for its robust glucose responsiveness, and therefore considered as the most physiologically relevant beta-cell model, were exposed to several mixtures containing different concentrations of 2,3,7,8-tetrachlorodibenzodioxin, di(2-ethylhexil)-phthalate, bisphenol A and PCB 153. After 1 or 24 hours exposure we evaluated: cell viability, glucose-stimulated insulin secretion, ultrastructural alterations and ROS production.

RESULTS

24 h exposure to a mixture containing low, non-cytotoxic concentrations of four different POPs, induces a significant decrease in the viability of INS 832/13 cells. 1 h exposure to POP mixtures at even lower concentrations, causes a significant impairment of glucose-stimulated insulin secretion. Electron microscopy highlights numerous ultrastructural alterations which also in this case are more severe in INS 832/13 cells exposed to POP mixtures than in those induced by exposure to single POPs. Finally, ROS production was significantly higher in INS 832/13 cells exposed for 1 or 24 h to a mixture of low concentrations of POPs compared to exposure to single pollutants.

CONCLUSIONS

Our results represent preliminary experimental contribution to understand how pollutants work together to produce combined toxicity and emphasize the need to take mixtures into account during chemical testing and risk assessment.

P068

INTEGRATING SALIVARY HPV GENOTYPING AND ORAL MICROBIOME ANALYSIS IN ORAL CAVITY SQUAMOUS CANCER (OCSCC)

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BACKGROUND-AIM

While persistent human papillomavirus (HPV) infection is an established risk factor for head and neck squamous cell carcinomas (HNSCC), its role in oral cavity cancer (OCSCC) remains less clearly defined. Early diagnosis of OCSCC is often hindered by the asymptomatic nature of early lesions and the complex anatomy of the oral cavity. Saliva-based liquid biopsy offers a non-invasive alternative for detecting oncogenic HPV genotypes and characterizing the oral microbiome, potentially improving early detection and risk stratification.

METHODS

In this retrospective study, 189 OCSCC patients and 55 healthy controls provided saliva samples, which were analyzed using a multiplex RT-qPCR assay (Seegene) targeting 28 HPV genotypes, and next-generation sequencing (Illumina 16S) for oral microbiota profiling.

RESULTS

HPV detection in saliva showed full concordance with tumor swab results (Cohen's kappa = 1), with 100% sensitivity and specificity. HPV DNA was found in 7.6% of patients, with genotype 58 being the most frequent (39.7%), followed by types 45, 59, and 39. HPV positivity was significantly associated with advanced TNM stage ($p \leq 0.001$) and with primary tumors compared to recurrences ($p = 0.04$).

Microbiota profiling revealed a distinct dysbiotic signature in OCSCC patients, characterized by an increased abundance of Bacilli (Firmicutes) and a reduction in Proteobacteria and Fusobacteria, associated with tumor-promoting inflammation and oral carcinogenesis. Notably, Veillonella abundance correlated with tumor stage, suggesting its potential role as a microbial biomarker of disease progression. A similar trend was observed for Streptococcus salivarius, whose decreasing abundance may reflect the loss of protective commensal taxa and a permissive environment for tumor development.

CONCLUSIONS

This study confirms the diagnostic reliability of salivary HPV testing and supports its potential use for non-invasive screening and monitoring of OCSCC. Additionally, the observed microbiota alterations suggest a possible link between dysbiosis, HPV infection, and carcinogenesis, warranting further investigation in larger, longitudinal cohorts.

P069

MITOCHONDRIAL BIOENERGETIC DYSFUNCTION AS INDICATOR OF FRAILTY: INSIGHTS FROM THE FRAMITO STUDY

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BACKGROUND-AIM

Frailty syndrome frequently coexists with multimorbidity, sharing several risk factors and clinical outcomes. The FRAMITO study, supported by the European Union – Next Generation EU (Mission 4-C2, CUP: 2022NSN355), aims to identify mitochondrial dysfunction biomarkers associated with frailty, both with and without multimorbidity.

METHODS

The FRAMITO cross-sectional study enrolled 82 individuals aged 65 and older from inpatient and outpatient clinics at the Geriatrics Units of the University Hospitals of Ferrara and Monza (Italy). Participants were categorized into three groups: 25 without frailty and multimorbidity (NFWoM), 25 with frailty but without multimorbidity (FWoM), and 32 with both frailty and multimorbidity (FWM). Peripheral blood samples were collected to isolate peripheral blood mononuclear cells (PBMCs) and their CD14 +, CD3+, and CD19+ subpopulations. Mitochondrial dysfunction was assessed via Seahorse assay, reactive oxygen species (ROS) quantification, and mitochondrial DNA (mtDNA) copy number variation.

RESULTS

Preliminary analysis comparing the FWM (median age: 87 years; 67.7% female), FWoM (median age: 83 years; 69.6% female), and NFWoM (median age: 72 years; 31.6% female) groups revealed a significant reduction in the Basal Respiration/Basal Glycolysis ratio in both CD14and CD3+ cells of the FWoM group compared to the NFWoM group. Notably, in CD14+ cells from the NFWoM group, a strong positive correlation was observed between ROS production and both basal respiration (R = 0.73) and mitoATP production rate (R = 0.77). This correlation weakened in the FWoM (R = 0.30 and 0.32) and FWM (R = 0.28 and 0.18) groups. These patterns suggest progressive mitochondrial dysfunction and oxidative stress in frail individuals, independent of multimorbidity.

CONCLUSIONS

Mitochondrial function in CD14+ cells declines with frailty. The reduced Basal Respiration/Basal Glycolysis ratio and weakened ROS correlation may indicate impaired energy metabolism and detox systems. CD14+ bioenergetic markers may serve as indicators of frailty, regardless of multimorbidity.

P070

REPURPOSABLE DRUGS MEBENDAZOLE AND NITROXOLINE IN COMBINATION EXHIBIT ANTIPROLIFERATIVE EFFECTS IN 2D AND 3D CELL MODELS OF PANCREATIC CANCER

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BACKGROUND-AIM

Pancreatic cancer (PC) is one of the most hard-to-treat tumors. Thus, more effective agents are needed to improve PC patient outcome. Drug repositioning is a valuable strategy in the search for already approved non-anticancer drugs that might be exploited in cancer treatment. Mebendazole (MBZ) and nitroxoline (NTX) are promising repurposable drugs that were active as single agents in many preclinical cancer models, including PC. Here, we evaluated the antiproliferative effects of MBZ and NTX in combination using 2D and 3D cell models of PC.

METHODS

Distinct PC cell lines were employed to assess antiproliferative effects of MBZ and NTX in combination. Viability and self-renewal capacity of PC cells after treatments were evaluated by MTT and clonogenic assays, respectively. Flow cytometry and immunoblot analyses of PARP cleavage and of proteins relevant for cell cycle were used for evaluating the impact of combined treatments on apoptosis and cell cycle distribution. Two PC-derived 3D models were also established to test the effects of MBZ/NTX combined treatments on organoid sizing and death.

RESULTS

We showed that some of the MBZ/NTX combined treatments significantly inhibited PC cell viability, compared to single agents, although with distinct sensitivities across the tested PC cell lines. The most sensitive PC cell lines, namely PATU8902 and L3.6Pl, were selected to further assess the antitumor effects of MBZ/NTX in combination. Specifically, the most active combination impaired clonogenicity, induced apoptosis and interfered with cell cycle progression in PC cells. Furthermore, MBZ/NTX in combination affected spheroids growth in ECM in both PC-derived 3D models, similarly to single agents. Notably, combined treatment was more effective than single agents in promoting cell death in L3.6Pl-derived 3D spheroids.

CONCLUSIONS

Our results show that mebendazole/nitroxoline in combination may expand the repertoire of agents to be exploited in the unsatisfactory PC treatment.

*These authors share first authorship.

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P071

MIMICKING BLOOD FLOW USING A NEAR IN VIVO MICROFLUIDIC SYSTEM TO STUDY CHEMICALS AND NANOMATERIALS (CNMS) METABOLIC RESPONSE

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BACKGROUND-AIM

To develop new methods which will enhance improvement of safety and sustainability assessments of chemicals and nanomaterials (CNMs), an EU funded research and innovation project “IMPLEMENTING INNOVATIVE METHODS FOR SAFETY AND SUSTAINABILITY ASSESSMENTS OF CHEMICALS AND MATERIALS PARTICULARLY AT NANO LEVEL IN THE EUROPEAN UNION (CheMatSustain)” is including 10 EU partners (and UK Associated partner). Related to safety assessments, an in vitro static condition has been adapted to dynamicity to mimic blood flow in a new microfluidic near in vivo model. The experimental approach started with physicochemical CNMs characterization (DLS, Z-Potential, AFM, HR-SEM/TEM - UV-VIS, FT-IR, SIMS, DSC, TGA, UPS/XPS and AI-Artificial Intelligence). EA.hy926 vascular endothelial cell has been used to set up the static experimental condition and has been translated to the dynamic microfluidic one. The microfluidic system consists of a pump that generates a physiological blood flow in chambers containing the endothelial cells in different CNMs treatments considtions.

METHODS

CNMs characterization has been supported by AI to profile the similarities and differences of the UPS/XPS photoelectron spectra analysed. The assessment of CNMs effects was performed considering cell viability, cell morphology, cell toxicity (ROS production) and inflammatory response (TLR signalling pathway activation: IRAK-1, IL-6 and miR-146a expressions).

RESULTS

Depending on physicochemical CNMs characteristics, our results demonstrate that viability, cell morphology and toxicity are differently affected in static related to dynamic conditions. This latter seems to protect CNMs from toxicity. CNMs can affect cell morphology in both models. Ongoing transcriptomics and inflammatory response analyses are evidencing mechanistic processes.

CONCLUSIONS

Considering that shear stress created by flow can maintain the quiescence and integrity of endothelial cells, the microfluidic near in vivo condition demonstrated that blood flow may protect from the CNMs cytotoxicity observed during static conditions. This innovative approach can mimic a cause-and-effect relationship between various types of blood circulating CNMs. Fluidic system for advanced cell culture allows better mimicking the in vivo environment and physiologically-like conditions.

P072

THERAPEUTIC LAV-BPIFB4 REVERSES VASCULAR IMPAIRMENT DRIVEN BY ADIPOSE INFLAMMATION IN APOE -/- FED HIGH-FAT DIET MICE

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BACKGROUND-AIM

Obesity triggers a chronic low-grade inflammatory state that contributes to cardiovascular and metabolic diseases. The over-release of adipokines and pro-inflammatory mediators by white adipose tissue (WAT) enhances inflammation through a feedforward loop involving endothelial and immune cells, and thereby promoting atherosclerosis. Our previous studies showed that in vivo gene transfer of the longevity-associated variant (LAV) of BPIFB4 restores endothelial and cardiac function, and reduces systemic inflammation burden in mice models. In this work, we investigated the anti-inflammatory potential of orally administered recombinant rhLAV-BPIFB4 in ApoE-/- fed a high-fat diet mice to elucidate its role in modulating endothelial dysfunction primed by adipose tissue inflammation.

METHODS

n=5 ApoE-/- mice subjected to standard diet (SD), n=5 (VEH-HFD) and n=6 (LAV-HFD) ApoE-/- fed high-fat diet mice in absence or presence of the rhLAV-BPIFB4 protein, respectively, have been considered. In addition to the analysis of circulating plasma cytokines levels, primary pre-adipocyte cultures were established from epididymal WAT to evaluate a) CD45+CD38+ leukocyte infiltration, b) the inflammatory profile of pre-adipocytes by analyzing key inflammatory pathways and cytokine release, and c) the ex vivo effect of conditioned media of pre-adipocytes on healthy and dysfunctional vessels. FACS analysis, Western Blotting, and Vascular reactivity studies were performed.

RESULTS

We found the oral administration of rhLAV-BPIFB4 in vivo in ApoE-/- fed high-fat diet mice dampens atherosclerosis by preserving endothelial integrity, reducing ICAM+ and CD68+ cell infiltration. Despite unchanged adiposity index, at systemic level rhLAV-BPIFB4 slows pro-inflammatory cytokines (IL-1α/β, TNF-α, IL-6) while midly increasing IL-10 levels. At tissue level, supernatants from pre-adipocytes from eWAT in ApoE-/- fed high-fat diet mice treated with rhLAV-BPIFB4 demonstrate analogous anti-inflammatory cytokine profile (low levels of IL-1α/β, TNF-α, IL-6). Accordingly, conditioned media from rhLAV-treated eWAT ex vivo restores endothelial function in dysfunctional arteries (VEH-HFD vs LAV-HFD, ***p<0.001, Two-way ANOVA).

CONCLUSIONS

Targeting adipocyte-associated inflammation, LAV-BPIFB4 emerges as a promising therapeutic strategy to counteract endothelial dysfunction in obesity.

P073

MODULATION OF INFLAMMATION BY THE LONGEVITY-ASSOCIATED BPIFB4 GENE: A PLATELET CD47-DEPENDENT SIGNALING

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BACKGROUND-AIM

Beyond their established function in haemostasis, platelets also play a crucial role in immunity and inflammation. In this context, platelets have been recently found to produce high levels of BPIFB4, a protein involved in preventing immune dysregulation. In line with its enrichment in Long-Living Individuals (LLIs), who exhibit stronger anti-inflammatory abilities, we have associated a polymorphic haplotype variant in the BPIFB4 gene (LAV-BPIFB4) with a reduced risk of cardiovascular complications and inflammation, thereby maintaining a healthy equilibrium between inflammatory and anti-inflammatory circuits. Additionally, recent studies showed that platelets and monocytes interact to mitigate inflammation through a CD47-dependent pathway. Therefore, our aim is to define a new mechanism of immune homeostasis which could reduce the harmful effects of inflammation in aged people.

METHODS

THP-1 monocytes were co-cultured with platelet-rich-plasma (PrP) from healthy donors and then treated with LPS to mimic an inflammatory microenvironment in vitro. The CD47 expression was analyzed by multiparametric flow cytofluorimetry in whole blood and on PrP. Protein levels were assessed by Western blotting; while the concentration of inflammatory cytokines were determined by ELISA. Unpaired T test and One way ANOVA test were applied to calculate statistical significance.

RESULTS

Immunophenotypical analysis revealed that CD41+/CD61+ platelets from homozygous LAV-BPIFB4 carriers exhibit higher CD47 expression (both as % of positive cells and as mean of fluorescence intensity) compared to those from wild-type (WT) donors. As consequence, LAV-derived platelets were more effective in restraining monocyte activation (as indicated by reduced IL-6 secretion after LPS stimulation). According to the immunomodulatory potential of LAV-genotype, donors PrP pre-conditioned with rhLAV-BPIFB4 (for 40' at 18ng/mL) showed a significant CD47 upregulation (****p<0,0001).

CONCLUSIONS

These findings a) support the emerging role of platelets as active immune regulators mitigating monocyte-driven inflammation and the importance of CD47 for platelet-monocyte interaction; b) suggest that changes in CD47 expression may reflect systemic inflammatory status, contributing to immune dysregulation; c) hint the enhanced CD47 expression on LAV-carriers platelets as a protective mechanism linked to this genotype, associated with immune homeostasis and reduced inflammation, potentially underlying healthy aging.

P074

ARSENIC TRIOXIDE IMPROVES THE SENSITIVITY TO IMMUNOTHERAPY IN NON-SMALL CELL LUNG CANCER

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BACKGROUND-AIM

Lung cancer is a major global health concern, with non-small cell lung cancer (NSCLC) representing the majority of cases and a significant challenge in clinical management. Despite multiple therapeutic approaches, the resistance to treatments remains a considerable impediment particularly in advanced-stage patients. Immunotherapy (e.g., anti-programmed cell-death protein 1/protein ligand 1, α-PD-1/α-PD-L1) has emerged as a promising approach in NSCLC treatment, either as monotherapy or combined with chemotherapy. However, primary and secondary resistance to immune checkpoint blockade reduces its efficacy through highly complex and still largely unexplored mechanisms. Arsenic trioxide (ATO) is known for its efficacy in acute promyelocytic leukemia and has emerged as a potential therapeutic agent in solid tumors by affecting multiple pathways.

METHODS

To explore the therapeutic potential of ATO in NSCLC, we adopted a multidisciplinary strategy combining in vitro assays with in vivo studies in syngeneic mouse models of NSCLC, further supported by advanced spatial proteomics analyses. These complementary approaches were designed to elucidate both the direct anticancer activity and the immunomodulatory impact of ATO, administered alone or in combination with immunotherapy.

RESULTS

ATO demonstrated potent anti-proliferative effects in both murine and human NSCLC cells in vitro. In vivo, ATO combined with α-PD-L1 treatment in a syngeneic immunocompetent mouse model of NSCLC led to a significant reduction in tumor growth and volume compared to either drug alone. Spatial proteomics profiling, focused on immune-related markers, revealed an increased infiltration of CD45+/Ly6G+ neutrophils in the tumor microenvironment following ATO treatment, suggesting a reprogramming of immune cell dynamics that may enhance the efficacy of immunotherapy.

CONCLUSIONS

Our findings identify ATO as a potent sensitizer of NSCLC to α-PD-L1 blockade. By modulating the tumor microenvironment and promoting a more immunogenic phenotype, ATO enhances the therapeutic response to immunotherapy. These results support the rationale for clinical evaluation of ATO in combination immunotherapy regimens, offering a promising strategy to overcome resistance and improve outcomes in NSCLC patients.

P075

GPR81 ACTIVATION DRIVES LACTATE-MEDIATED BCR/ABL SUPPRESSION IN CHRONIC MYELOID LEUKEMIA CELLS UNDER HYPOXIA

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BACKGROUND-AIM

Chronic myeloid leukemia (CML) is a stem cell–driven neoplasia characterized by the expression of the constitutively active BCR/Abl tyrosine kinase (TK). Within the low-oxygen environment of stem cell niches (SCN) in vivo, the oncogenic BCR/Abl protein is suppressed. Consequently, Leukemia Stem Cells (LSC) residing within SCN show resistance to TK inhibitors (TKi), the first-line therapy for CML, due to the lack of their molecular target. It is therefore critical to elucidate the mechanisms driving BCR/Abl protein suppression under low oxygen conditions to develop strategies to overcome TKi resistance. Our previous studies showed that BCR/Abl protein is suppressed when glucose approaches complete exhaustion in culture medium. Given that lactate is the principal by-product of glucose metabolism under hypoxia, we investigated its potential role in modulating BCR/Abl protein expression.

METHODS

K562 and KCL22 stabilized CML cell lines were cultured for 5/7 days in low oxygen atmosphere (0.1% O2) cultures (LC1). Cells were treated with lactate and/or lactate transporter inhibitors, and/or GPR81 agonist or antagonist. At the end of LC1, BCR/Abl protein expression and intra-/extra-cellular lactate concentrations were determined. To estimate stem cell potential, cells were transferred to normoxic secondary cultures (LC2) and their repopulation capacity measured.

RESULTS

Lactate transporter inhibition in LC1, which caused reduced lactate excretion, led to the maintenance of BCR/Abl expression and a faster LC2 repopulation. These effects were reverted when exogenous lactate was added to the culture medium, resulting in the suppression of BCR/Abl protein expression. Since accumulated extracellular lactate can function as a signalling molecule, by binding to the lactate receptor GPR81, we evaluated the involvement of GPR81 in the modulation of BCR/ABl expression. We found that the treatment with GPR81 antagonist 3-OBA prevented BCR/Abl suppression, while the treatment with the GPR81 agonist CHBA counteracted the MCT inhibitor-induced maintenance of BCR/Abl protein and signalling.

CONCLUSIONS

Our results indicate that extracellular lactate accumulation determines BCR/Abl protein suppression in low oxygen through GPR81 activation.

P076

MOLECULAR ALTERATIONS IN A COHORT OF RECTAL CANCER PATIENTS POINTED TO ITS INDEPENDENT TUMOUR ENTITY

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BACKGROUND-AIM

Most previous studies have investigated colorectal carcinomas without making a specific distinction between carcinomas originating from the colon (colon carcinomas, CC) and those originating from the rectum (rectal carcinomas, RC), as the large bowel has always been considered as a single organ. For this reason, we aimed to perform a molecular characterisation of rectal cancers and a comparison with the molecular profiles of colon cancer samples (analysed in previous studies) to identify rectum-specific alterations

METHODS

Twenty surgically removed rectal cancer samples were subjected to NGS analysis to identify mutations, gene copy number alterations (CNAs) and activated signalling pathways. Immunohistochemical, survival and TCGA analyses were performed to validate our results in a larger cohort of patients

RESULTS

We identified 225 potentially protein-altering somatic variants in 111 genes and 598 copy number alterations (CNAs) in 341 genes. The most frequently mutated genes included TP53 (60%), APC (45%), TET2 (45%), PDE4DIP (40%) and DCC (35%), which are common in rectal and colon cancers. However, some other mutated genes may represent new drivers of cancer development in the rectal epithelium: BLM (10%), MRE11 (5%), RECQL4 (5%). The analysed rectal carcinomas showed stable microsatellites (and proficient MMR) and a higher average number of CNAs. Patients with mutations in NER, FA and HR DNA repair pathways had higher mean CNA values (median=47, range 12-125) than patients without mutations (median=5, range 0-7). The chromosomal loci that most frequently showed CN gains in RCs were 6q23.2-23.3, 7q21-22, 7q36, 12q12, while those with CN losses were 1p36.31, 2p25.2, 12q24.33. Finally, Ingenuity Pathway Analysis helped us to define signalling pathways that were enriched in rectal carcinomas compared to colon carcinomas: G protein-coupled receptor signalling pathway, Cancer metastasis, FAK signalling pathway, MSP-RON signalling pathway, general xenobiotic metabolism signalling pathway.

CONCLUSIONS

Our results suggest that rectal and colon cancers have significant molecular differences that certainly deserve further investigation to identify specific biomarkers that can be used for targeted therapeutic strategies.

P077

MODERATE TRAINING PREVENTS DIET INDUCED CARDIOVASCULAR REMODELING IN FEMALE LONG-EVANS RATS

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BACKGROUND-AIM

Western-style diet and sedentary life are important risk factor of obesity leading to cardiac hypertrophy and fibrosis. The purpose of this study is investigating the effects of diet and training on cardiac remodeling.

METHODS

48 female Long-Evans rats were divided into six groups: a control (CTRL) group, a solid high fat (HF) group, a combined solid high fat and sugar water diet (HFHS) and the respective trained groups (CTRLtrain, HFtrain, HFHStrain). Rats were trained on treadmill at 10% slope for 30 minutes 4 days a week for 8 months. Diet and training started at the 5th month of age. Echocardiography was carried out one month before study end. Rat Hearts were formalin fixed for Masson's trichrome and immunohistochemistry.

RESULTS

AWTd increased with training (CTRL vs HFtrain mean diff. = -0,3417 mm, $p<0,05$; CTRL vs HFHStrain mean diff.= -0,3222 mm, $p<0,05$; HF vs HFtrain mean diff.= -0,3306 mm, $p<0,05$) as well as PWTd, AWTs, PWTs and LV mass (CTRL vs HFtrain mean diff. = -0,2275 g, $p<0,01$; CTRL vs HFHStrain mean diff.= -0,1978 g, $p<0,05$; HF vs HFtrain mean diff.= -0,2097 g, $p<0,01$; HFHS vs HFHStrain mean diff.= -0,1711 g, $p<0,05$). EF% and SF% were unchanged but E wave velocity improved with training (CTRL vs HFtrain mean diff. = -0,3238 ms, $p<0,05$; HF vs HFtrain mean diff.= -0,2873 ms, $p<0,001$; HFHS vs HFHStrain mean diff.= -0,1838 ms, $p<0,05$).HF showed significant fibrosis which was reversed by training (CTRL vs HF mean diff. =-1,376 %, $p<0,001$; CTRL vs HFtrain mean diff. = 1,356 %, $p<0,01$; CTRL vs HFHStrain mean diff.= 1,437%, $p<0,001$; HF vs CTRLtrain mean diff.= 1,979% $p<0,001$, HF vs HFtrain mean diff.= 2,732 %, $p<0,001$; HFHS vs HFHStrain mean diff.= 1,622 %, $p<0,001$). No changes in myocyte CSA, capillary or coronary arteries density were found however artery wall/lumen ratio decreased with training (CTRL vs CTRLtrain mean diff.= 0,05596, $p<0,05$ HF vs CTRLtrain mean diff.= 0,07386 $p<0,01$, HF vs HFtrain mean diff.= 0,06143, $p<0,05$; HFHS vs CTRLtrain mean diff.= 0,05904, $p<0,05$).

CONCLUSIONS

In conclusion HF diet increased fibrosis, moderate training induces heart wall thickening in absence of cardiomyocyte hypertrophy and improves diastolic function as well as preventing fibrosis and decreasing coronary wall thickening.

P078

THE EFFECTS OF TRPM8 MODULATORS IN MELANOMA CELL DEATH: A NEW WEAPON

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BACKGROUND-AIM

Cutaneous melanoma is a highly aggressive malignancy with a rising global incidence. Although targeted therapies and immunotherapies have improved patient outcomes, their efficacy is often limited by toxicity and the development of resistance.

Therefore, novel therapeutic strategies are urgently needed. The transient receptor potential melastatin subtype 8 (TRPM8), a non-selective cation channel with a preference for calcium permeation, is aberrantly expressed in various solid malignancies, including melanoma, and may represent a promising therapeutic target.

This study aims to investigate the antineoplastic effects of novel TRPM8 modulators as potential therapeutic agents for melanoma, to overcome the limitations of current treatments.

METHODS

Two melanoma cell lines, derived from subcutaneous and lymph node metastases, were used to evaluate the effects of TRPM8 modulation. Multiple methodologies, including colorimetric assays, western blotting, immunofluorescence (IF), and confocal microscopy, were employed to assess cellular responses to TRPM8 modulators.

RESULTS

Treatment with TRPM8 modulators resulted in a significant, dose- and time-dependent reduction in melanoma cell viability at micromolar concentrations, without inducing cytotoxicity in normal fibroblasts used as controls. Mechanistically, treatment with TRPM8 modulators led to the accumulation of reactive oxygen species (ROS), triggering apoptosis.

Furthermore, TRPM8 modulation disrupted mitochondrial function, as evidenced by $\Delta\psi$m collapse, cytochrome c release, and caspase-3 activation. Notably, siRNA-mediated knockdown of TRPM8 abrogated these effects, confirming the specificity of TRPM8-targeted action. Additionally, TRPM8 modulators significantly reduced the growth of 3D spheroids.

CONCLUSIONS

Further studies are warranted to elucidate the molecular pathways involved in TRPM8 mediated-cell death. Nevertheless, the identification of TRPM8 as a selective and potent target highlights its potential for the development of innovative therapeutic strategies in melanoma. These findings contribute to the growing field of precision oncology and support the advancement of TRPM8 modulators as promising anti-melanoma agents.

P079

TERRA AS A SENESCENCE AND PROGNOSTIC BIOMARKER IN PERIPHERAL BLOOD MONONUCLEAR CELLS: FINDINGS FROM COLORECTAL CANCER PATIENTS AND IN VITRO STUDIES

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BACKGROUND-AIM

Aging is a significant risk factor for major diseases such as cancer. A multiparametric aging profile assessed in peripheral blood—covering immune senescence, telomere length, thymic output, circulating senescence-associated secretory phenotype (SASP), and denervation/sarcopenia markers (CAF, NCAM1)—offers a minimally invasive approach to monitor biological aging and outcomes in elderly colorectal cancer (CRC) patients. A key emerging biomarker is TElomeric Repeat-containing RNA (TERRA), a class of long noncoding RNAs transcribed from telomeres that affect telomere dynamics and cellular senescence, although its role under senescence-inducing conditions remains unclear.

METHODS

We analyzed 70 elderly CRC patients at surgery, measuring TERRA transcripts from chromosomes 1q-2q-10q-13q (TERRAch1-2-10-13), 15q (TERRAch15), 20q (TERRAch20), and XpYp (TERRAchXY) by qRT-PCR, alongside other aging biomarkers: T-cell immunophenotyping (flow cytometry), thymic output and telomere length (qRT-PCR), denervation and sarcopenia markers (ELISA), SASP factors (Luminex). All parameters were correlated with clinical outcomes.

RESULTS

An aged phenotype—characterized by high levels of senescent and activated CD8 T cells, elevated circulating SASP markers (IL-6, IL-8, CXCL-1) and CAF levels—was associated with worse outcomes. Patients with high TERRAch15 levels had fewer senescent CD8 T cells ($p=0.014$), higher CD4/CD8 ratios ($p=0.038$), and lower risk of adverse events (HR: 0.37, $p=0.028$).

To explore TERRA dynamics under senescence-inducing conditions, we treated peripheral blood mononuclear cells (PBMCs) from healthy donors with doxorubicin (40 nM, 72 h) or telomerase inhibitor BIBR1532 (20 μM, 11 days). Doxorubicin induced a senescence-like phenotype with a trend in telomere shortening ($p=0.114$), upregulation of CDKN1A ($p=0.002$) and CDKN2A ($p<0.001$), downregulation of LMNB1 ($p=0.001$), and increased TERRA levels (TERRAch1-2-10-13, $p=0.016$; TERRAch15, $p=0.006$; TERRAch20, $p<0.001$; TERRAchXY, $p<0.001$). Similarly, BIBR1532-treated PBMCs showed a senescence profile with increased CDKN1A ($p=0.011$), CDKN2A ($p=0.034$), reduced LMNB1 ($p=0.023$), and elevated TERRA (TERRAch1-2-10-13, $p=0.040$; TERRAch15, $p=0.017$; TERRAch20, $p=0.090$; TERRAchXY, $p=0.003$).

CONCLUSIONS

These findings indicate that a blood-based aging signature is prognostic for CRC patients, and highlight TERRA as a potential marker of biological aging. Its upregulation in response to senescence stresses suggests a role in aging defense processes.

P080

ROLE OF RNA-BINDING PROTEIN AU-RICH-ELEMENT FACTOR-1 (AUF-1) IN HUMAN AIRWAY EPITHELIAL SENESCENCE AND INFLAMMATORY RESPONSE

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BACKGROUND-AIM

The RNA-binding protein AU-rich-element factor-1 (AUF-1) is a posttranscriptional regulator (PTR) of inflammatory responses and genomic integrity by preventing cellular senescence. AUF-1 binds to target mRNAs via conserved sequences and to other proteins as ribonucleoprotein (RNP) complexes, regulating mRNA decay and translation of cell-cycle checkpoint and senescence-associated secretory phenotype (SASP) factors. We described a loss of AUF-1 expression in vivo, in bronchiolar epithelium of COPD patients vs. matched controls and in vitro, in cytokine-stimulated human airway epithelial cells. This was associated with increased epithelial senescence and SASP and AUF-1 enrichment in extracellular vesicles (EVs). We hypothesized that AUF-1-bound proteins could contribute to its regulatory function and that AUF-1-containing RNP acts as cargo for bound transcripts to EVs; thus, we aim at characterizing AUF-1 protein interactome and EVs proteome in human airway epithelial BEAS-2B cells.

METHODS

Immunoprecipitation (IP) of protein extracts from resting cells was performed with anti-AUF1 Ab (Atlas Ab). Moreover, BEAS-2Bs with CRISPR-Cas9 ablation of HNRNPD gene coding for AUF-1 and control cells were cultured in EVs-free medium alone or with cytomix (48 h, 10 nM each TNFα+IL1β+IFNγ). EVs were isolated by ultracentrifugation. Proteins from both protocols were digested, analyzed by nano LC-MS/MS and characterized bioinformatically (STRING, ShinyGO).

RESULTS

We identified putative AUF-1 protein partners (n=14), including AUF-1 family members HNRPC1/C2 and HNRPM, with functional prominence in RNA metabolism including mRNA binding and enzymatic activity, and functions related to extracellular region and vesicles (n=9). EVs proteome also contained AUF-1 partners, with involvement in RNA metabolism and translation (n=107) and intracellular transport (n=141). Cytomix stimulated, AUF-1 competent EVs were enriched in proteins involved in stress response, a function partially lost in AUF-1 KO stimulated EVs which maintained a prominence in PTR functions, such as protein localization to cytoplasmic stress granules.

CONCLUSIONS

Composition and inflammation-driven changes in AUF-1 RNP and its localization to EVs may control bound transcripts selected for EVs transfer and influence global epithelial functions.

P081

α-KETOGLUTARATE–DRIVEN IMMUNE ESCAPE IN HYPOXIC MASLD-HCC: AMINO ACID METABOLISM AT THE CROSSROADS

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BACKGROUND-AIM

Metabolic dysfunction-associated steatotic liver disease (MASLD)-related hepatocellular carcinoma (HCC) is an emerging global health concern, driven by the rising prevalence of obesity, type 2 diabetes, and metabolic syndrome. As MASLD progresses to HCC, profound alterations in the tumor microenvironment (TME) contribute to tumor aggressiveness and immune evasion. Hypoxia is a defining feature of the TME and drives metabolic reprogramming, including increased utilization of branched-chain amino acids (BCAA) and glutamine, which fuel tumor growth and generate oncometabolites that potentially reshape the tumor immune landscape. This study explores how BCAA and glutamine catabolism affect pro-tumoral macrophage polarization and immune evasion.

METHODS

This study was performed on: a) cohorts of MASLD/MASH-HCC patients; b) murine models for disease progression from MASLD (CDAA diet) to MASH-related HCC (DEN/CDAA protocol) with hepatocytes deletion for HIF-2α (HIF-2α-/-); c) in vitro experiments on HepG2 and human THP-1 macrophages.

RESULTS

Our findings indicate that hypoxic conditions upregulate the expression of the BCAA transporter LAT1 and the glutamine-metabolizing enzyme glutaminase 1 (GLS1) in both in vitro and in vivo models and patient-derived MASH-HCC tissues, with their expression levels being positively associated with hypoxia-inducible factor 2α (HIF-2α). In the MASLD-HCC experimental model, elevated serum BCAA levels and increased hepatic expression of catabolic enzymes (BCAT1/2, GLS1/2, GLUD) were observed, while hepatocyte-specific HIF-2α knockout markedly reduce their levels. Activation of these pathways, alongside altered regulation of TCA-cycle enzymes, likely drives α-ketoglutarate (α-KG) accumulation, promoting M2-like polarization of macrophages, potentially contributing to immune evasion within the TME.

CONCLUSIONS

In summary, hypoxia-induced reprogramming of BCAA and glutamine metabolism in MASLD-related HCC contributes to immune evasion via oncometabolite-driven mechanisms. Targeting these metabolic nodes may offer new therapeutic strategies to restore anti-tumor immunity in this increasingly prevalent cancer subtype.

P082

CIRCULATING MIRNAS PROFILING IN IDIOPATHIC PULMONARY FIBROSIS

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BACKGROUND-AIM

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive interstitial lung disease, characterized by irreversible fibrotic remodeling. Its pathogenesis still remains incompletely understood. Early diagnosis and treatment are crucial to slow disease progression. Circulating microRNAs (ci-miRNAs) have emerged as promising non-invasive biomarkers. This study aimed to characterize plasma ci-miRNA profiles in patients with early and established IPF and compare them to healthy controls, to identify specific signatures and pathways of disease onset/progression.

METHODS

Plasma samples were collected from 11 early IPF patients, 14 patients with established IPF, and 11 healthy controls. Total RNA was subjected to RNA-Seq. Differential expression was assessed by DESeq2. Functional enrichment of predicted miRNA targets was assessed through GO and KEGG databases; hierarchical clustering evaluated group-specific expression patterns.

RESULTS

20 ci-miRNAs were differentially expressed in early IPF compared to healthy controls, all significantly downregulated. These did not overlap with miRNA signatures from fibrotic or systemic autoimmune conditions. Functional enrichment highlighted pathways related to cytoskeletal remodeling, GTPase-mediated signaling, and cell–matrix adhesion. KEGG analysis also showed enrichment for “non-small cell lung cancer”. In the comparison between early and established IPF, 236 ci-miRNAs were differentially expressed, with significant enrichment in fibrotic processes such as “keratin filament” and “supramolecular complex”. Hierarchical clustering clearly separated the three groups, with early IPF patients clustering closer to controls, indicating a progressive shift in ci-miRNA expression. This strong discriminatory capacity underscores the potential of ci-miRNAs as biomarkers to stratify disease stage.

CONCLUSIONS

This study reveals distinct stage-specific ci-miRNA profiles in IPF, with discriminatory power between healthy individuals and patients at different disease stages. This capacity to differentiate early from established forms of IPF has strong clinical relevance, supporting the use of ci-miRNAs as accessible biomarkers to improve diagnostic accuracy, guide early therapeutic interventions, and monitor disease progression.

P083

TARGETING BLADDER CANCER AGGRESSIVENESS: AN IN VITRO STUDY OF THE EFFECTS OF A MULTI-STRAIN PROBIOTIC FORMULATION LYSATE

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BACKGROUND-AIM

Bladder cancer (BC) is the most common type of malignancy affecting the urinary tract and is known for its high rates of recurrence and progression, particularly in high-grade forms. Recent studies have highlighted the potential of probiotics as supporting agents in cancer treatment due to their anti-cancer properties. Here, we investigated the effects of a multi-strain probiotic formulation (OxxySlab®) on two human BC cell lines with different degrees of malignancy (T24 and 5637), as well as on non-tumorigenic urothelial cell line SV-HUC-1.

METHODS

Cell proliferation, cell number, and cell cycle profile were evaluated using IncuCyte® Live Cell Imager system, trypan blue dye exclusion test, and flow cytometry. Additionally, the clonogenic potential through colony formation assay and migratory ability using in vitro scratch assay were assessed. We analyzed markers of epithelial-mesenchymal transition (EMT), including E-cadherin, vimentin and β-catenin, as well as the generation of intracellular ROS using a DCFH-DA probe. The cell senescence was evaluated through β-galactosidase staining and by measuring p53 and p16 expression.

RESULTS

Treatment with the multi-strain probiotic formulation led to a dose-dependent reduction in proliferation, colony formation, and migration in cancer cells in a statistically significant manner in both BC cell lines, while normal urothelial cells remained unaffected. Western blot analysis showed that exposure to probiotic significantly increased the levels of the epithelial marker E-cadherin, while decreasing the mesenchymal markers vimentin, and β-catenin, indicating an inhibition of EMT. The probiotic also elevated ROS levels, which promoted telomere shortening and cellular senescence in cancer cells, as evidenced by cell cycle arrest and increased β-galactosidase activity. In addition, the increased expression of p53 and p16 confirmed the activation of cellular senescence pathways in the BC cell lines.

CONCLUSIONS

Overall, our results underscore the potential of a multi-species probiotic formulation as a targeted anti-tumor strategy that effectively addresses critical malignant features of BC cells while sparing healthy urothelial cells. This approach may offer a promising adjuvant therapy in the clinical management of BC.

P084

EXPLORING L-ASPARAGINASE TREATMENT RESPONSE OF RS4;11 CELLS AS AN IN VITRO MODEL OF ACUTE LYMPHOBLASTIC LEUKEMIA THROUGH SCRNASEQ ANALYSIS

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BACKGROUND-AIM

Identifying new cancer and drug-resistance biomarkers is essential for improving prognosis and enabling effective therapies. Single-cell RNA sequencing (scRNA-seq) provides a powerful tool to dissect transcriptional heterogeneity at single-cell resolution. In this study, we investigated the transcriptional response to L-asparaginase (ASNase) in RS4;11, a cell line model of Acute Lymphoblastic Leukemia (ALL).

METHODS

RS4;11 cells (ATCC) were cultured in suspension and treated for 72h with ASNase (0 U/ml CTRL; 0.001 U/ml treated sample, N=3). Single-cell gene Expression Flex (10X Genomics) was then performed at the Italian National Facility HumanTechnopole. CellRanger v9.0.0 pipelines were used to convert raw sequencing data into FASTQ files and compute Gene-feature barcode matrices. Downstream analyses were performed in R through: DESeq2 v.1.46.0 and Seurat v.5.2.1 for differential gene expression (DGE) analysis; fgsea v.1.32.4 and clusterProfiler v.4.14.6 for gene set expression analysis (GSEA) exploiting Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

RESULTS

DGE analysis across all cells identified 4339 differentially expressed genes, including 505 upregulated (log2FC>1.5) and 635 downregulated (log2FC<-1.5), indicating a transcriptional response to treatment. Besides DGE of canonical genes involved in asparaginase response (e.g., AKT1, E2F1, E2F2, MYBL2, CCNE1, CCNE2, CDC6, FOXM1), we identified strong evidence of a ferroptosis transcriptional signature and in particular, upregulation of hallmark genes like CHAC1, PTGS2, and SLC7A11 and downregulation of antioxidant and protective genes like MT1G and TFRC. At the same time, suppression of anabolic fatty acid metabolism, with no clear signs of catabolic activation suggests reduced lipid synthesis.

CONCLUSIONS

RS4;11 cells exhibit a typical response to ASNase treatment with a high sensitivity. To identify the primary pathways involved in drug response that were not fully explained or known, we analyzed the scRNAseq signature of treated cells. We found a significant transcriptional pattern that suggests activation of ferroptosis and inhibition of lipid synthesis. This information has relevant translational potential, identifying new possible vulnerabilities to be exploited for ASNase treatment besides canonical ones.

P085

TNF-α IN MULTIPLE SCLEROSIS: INTEGRATED ANALYSIS OF MIRNA–MRNA AND PROTEIN EXPRESSION

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BACKGROUND-AIM

Multiple Sclerosis (MS) is a chronic and multifactorial disease characterized by the breakdown of the protective covering of nerves triggered by complex gene-environment interactions. Despite the well-known contribution of TNF-α to MS, several important knowledge gaps remain that prevent a full understanding of its precise roles in MS onset and progression.

METHODS

We performed an integrated analysis of the potential mechanisms regulating TNF-α in MS patients.
ELISA assay measured TNF-α levels in serum and saliva, while qPCR was used to assess its gene
expression in PBMCs isolated from patients and healthy controls. Changes in DNA methylation of the TNF-α have been observed by pyrosequencing reaction on genomic DNA. Bioinformatic tools TargetScanHuman 8.0 (https://www.targetscan.org/vert_80/) and miRTargetLink 2.0 (https://ccb-compute.cs.uni-saarland.de/mirtargetlink2/) were applied to the analysis of TNFα target miRNAs.

RESULTS

Quantification of TNF-α levels, both serum and saliva, showed a significant increase in MS patients compared to HC, with notably higher levels detected in saliva. Since circulating TNF-α reflects the combined output of multiple sources and is influenced by clearance rates, the assessment of TNF-α gene expression in PBMCs underline a significantly higher gene expression level in MS patients compared to HC. Hypothesizing an epigenetic mechanism of TNF-α expression regulation, we calculated with PyroMark CpG software the average Methylation Index (MI), displaying a significant reduction of methylation in the TNF-α promoter of MS patients compared to HC. Bioinformatic analysis led to the identification of miR-130a-3p as a highly significant regulator of TNF-α (score = 98), acting as a negative regulator of its expression. miR-130a-3p expression levels in serum and saliva were lower in MS patients than in HC.

CONCLUSIONS

In summary, in our patients, the epigenetic regulation of TNF-α gene expression and production occurs in both serum and saliva, suggesting that saliva could serve as a valuable diagnostic fluid for MS. Further research involving more individuals with MS is planned to validate the preliminary results.

P086

THE METABOLOMIC PROFILE OF PSORIATIC ARTHRITIS PATIENTS UNVEILS THE UNBALANCE OF DISEASE-RELATED MOLECULES AND PATHWAYS

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BACKGROUND-AIM

Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory disease characterized by marked clinical heterogeneity, stemming from the variable involvement of multiple disease domains, including cutaneous and nail psoriasis, peripheral arthritis, enthesitis, dactylitis, axial involvement (spondylitis), and sacroiliitis (Ritchlin, C.T. et al. 2017). PsA affects both sexes equally and is frequently associated with progressive structural joint damage, functional impairment, and a significant reduction in health-related quality of life. Inadequate or delayed therapeutic intervention can result in irreversible joint destruction and long-term disability (McHugh, N.J. et al. 2003). This study aimed to identify reliable and validated biomarkers needed to enhance diagnostic accuracy and facilitate the longitudinal monitoring of disease activity and progression. Such biomarkers would be pivotal in guiding individualized therapeutic strategies, thereby optimizing clinical outcomes and improving the overall quality of life for patients affected by PsA.

METHODS

The plasma metabolomic profile of 29 individuals with active psoriatic arthritis, defined by a DAPSA score >14 (no bDMARD treatment in progress), was analyzed and compared to that of 32 healthy controls (HC). Plasma samples were analyzed using a 1H-NMR spectroscopy-based approach, and the resulting data were processed through multivariate statistical analysis (MVA).

RESULTS

The multivariate statistical analysis showed a great separation between PsA and HC, indicating differences in the metabolomics profile between the two groups. Statistically significant metabolites that distinguished PsA from HC were involved in several metabolic pathways such as glucose-alanine cycle and glycine and serine metabolism, glutathione metabolism, as well as selenoaminoacid metabolism, alanine metabolism and tryptophan metabolisms.

CONCLUSIONS

Despite the limitation of this study due to a small cohort of subjects, our findings suggest that the metabolic differences in PsA patients compared to healthy conditions contribute to elucidating the role of metabolite unbalance on the inflammatory burden, oxidative stress, energy and collagen metabolism but also on peculiar features of PsA disease, as metabolic disorder and diabetes.

P087

FUNCTIONAL INTERACTIONS BETWEEN Y537S MUTANT BREAST CANCER CELLS AND ADIPOCYTES AS MUTUAL PLAYERS SUSTAINING TUMOR GROWTH AND PROGRESSION

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BACKGROUND-AIM

Although the endocrine therapy (ET) has significantly reduced breast cancer (BC) recurrence and mortality, somatic mutations in the estrogen receptor (ESR1) are one of the main mechanisms for the development of resistance to ET. Among these, the Y537S, in the ligand-binding domain of ERα, maintains the receptor in a constitutively active state, contributing to clinical phenotype, leading to tumor recurrence and distant metastases. Tumor progression is also shaped by the tumor microenvironment (TME), especially adipocytes, which represent important breast stromal component. During early tumor invasion, direct contact of cancer cells with adipocytes leads to their transformation into cancer-associated adipocytes (CAAs), which release hormones, cytokines, and proteases that support tumor growth. In the present study, we investigated at first how Y537S-mutant BC cells interact with adipocytes to modulate their phenotype differentiation and tumor-supportive functions. Then we deeply explored how unaltered adipocytes may affect tumor progression, migration, and invasiveness.

METHODS

We conducted coculture experiments with Y537S CRISPR-expressing MCF-7 BC cells (YS1), pre-adipocytes murine 3T3-L1 cells and differentiated adipocytes 3T3L1-A. Then, we tested the effects of BCs-CM on adipocytes cell proliferation (Anchorage-independent Soft Agar), cell motility and invasiveness (Transwell Migration and Invasion Assay). Protein and mRNA expressions was evaluated, respectively, by Western Blotting (WB) Assay and Real Time RT-PCR. Trasmission Electron Microscopy (TEM) was used to identify autophagic vacuoles.

RESULTS

We found that the exposure to YS1 conditioned medium (CM), exhibiting a higher amount of insulin growth factor-1 (IGF-1), influences autophagy in 3T3L1 pre-adipocytes and the de-differentiation process leading to CAA phenotype in a higher extent respect to the parental cells enhancing the invasiveness and supporting resistance to ET. Vice versa the 3T3L1A-CM influence and promote the proliferation, migration and invasiveness of Y537S mutant BC cells.

CONCLUSIONS

Our data evidence the bidirectional interaction between Y537S BC cells and surrounding adipocytes, shedding light on the role of the TME in endocrine resistance and tumor progression.

P088

METFORMIN IMPAIRS GLIOBLASTOMA GROWTH BY MODULATING THE FOXO3A/SURVIVIN SIGNALING AXIS

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BACKGROUND-AIM

Glioblastoma multiforme (GBM) is the most prevalent and aggressive primary brain tumor in adults, characterized by diffuse infiltration, rapid progression, treatment resistance, and poor survival outcomes. The limited efficacy of surgical resection along with rapid recurrence driven by therapy-resistant cells, underlines the identification of innovative therapeutic strategies as the most important challenge for the management of GBM.
Metformin (MET), an anti-diabetic agent, has recently emerged as a repurposable drug with a prominent anti-tumor activity in various malignancies, including GBM.
To better understand the molecular mechanism/s through which MET impairs GBM growth, we investigated MET ability to affect the expression and function of survivin, a multi-functional inhibitor of apoptosis (IAP) over-expressed in GBM and strongly implicated in therapy-resistance and tumor relapse.

METHODS

Experimental models: U87-MG and T98G cell lines; U87-MG-derived orthotopic xenografts. Cell viability/proliferation assays: MTT/Trypan Blue Exclusion/Anchorage-dependent and -independent Growth Assays. Gene/protein expression: qRT-PCR; WB Analysis; IHC; ChIP Assay; siRNA-Interference. Cell motility/invasiveness: Wound-healing/Boyden chamber transmigration/Matrigel invasion/Gelatin Zymography Assay; Phalloidin staining.

RESULTS

Here we report the AMPK/FoxO3a/survivin pathway as a critical node of vulnerability in GBM. MET treatment, in vitro, activates AMPK and promotes FoxO3a nuclear translocation, resulting in transcriptional repression of survivin, a key mediator of mitotic regulation, apoptosis, invasiveness and therapy resistance in GBM. Concomitantly, functional assays revealed that MET significantly reduces cell viability, proliferation and invasiveness. In vivo, MET treatment in U87-MG-derived orthotopic xenografts led to sustained FoxO3a upregulation and survivin downregulation, corroborating the in vitro findings and highlighting the translational relevance of this axis.

CONCLUSIONS

These results provide preclinical evidence that MET-dependent dual modulation of FoxO3a and survivin might offer potential promising avenues for developing more effective therapies and improve GBM patient clinical outcome.

P089

HUMAN ACTIVATED MACROPHAGES INDUCE GLUTAMINE SYNTHETASE AND THE EFFLUX GLUTAMINE TRANSPORTER SNAT5: A METABOLIC CROSS TALK WITH LYMPHOCYTES?

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BACKGROUND-AIM

L-Glutamine (Gln) plays a pivotal role in the activation of macrophages and lymphocytes. Macrophages express Glutamine Synthetase (GS, gene GLUL) and produce Gln. On the other hand, Gln availability is needed by $CD4^+$ and $CD8^+$ lymphocytes for full functional activation. In both cells, studies have been focused on Gln metabolism, while little attention has been thus far devoted to the role of Gln transmembrane fluxes and to possible metabolic cross talks between macrophages and lymphocytes. Objective of this study is to demonstrate that activated human macrophages can synthesize and extrude Gln into the extracellular medium.

METHODS

Primary monocyte-derived macrophages (MDM) and human THP-1 macrophage-like cells were exposed to proinflammatory M1-polarizing stimuli (LPS/IFNγ). Expression of Gln-related enzymes and transporters was assessed at both mRNA and protein level. Intracellular and extracellular levels of Gln and related amino acids were determined with LC-MS/MS. Gln membrane transport was determined with an original method based on the influx of [3^H]-Gln.

RESULTS

Long-lasting stimulation (24-48h) with LPS/IFNγ led to a marked induction of GS and of several Gln transporters, such as SNAT2 (SLC38A2), ASCT2 (SLC1A5) and SNAT5 (SLC38A5). In parallel, Gln uptake increases but the intracellular content of the amino acid does not appreciably change, while the consumption of extracellular Gln is slower. The characterization of Gln transport demonstrates a large stimulation of the activity of the efflux transporter SNAT5. These results suggest that M1 polarized human macrophages produce and secrete Gln. Preliminary experiments, performed with a triple co-culture of MDM/$CD8^+$ lymphocytes/bacteria, have indicated that specific bacterial strains stimulate IFNγ secretion by autologous $CD8^+$ lymphocytes and that, under these conditions, GS and SNAT5 are induced.

CONCLUSIONS

On the basis of these data, we hypothesize that activated human MDM secrete Gln, thus potentially modifying its availability in the immune microenvironment and influencing lymphocyte activity. If this is the case, the pharmacological interference with this metabolic cross talk would hinder $CD8^+$ cell activity, providing an immunometabolic tool to modulate lymphocyte overactivation.

P090

GLUTAMINASE-1 INHIBITOR CB-839 DETERMINES METABOLIC SHIFTS IN COLORECTAL CANCER CELLS

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BACKGROUND-AIM

To meet high energetic and biosynthetic demand, cancer cells adapt by prioritizing various energy sources. Several tumor cells, including colorectal cancer (CRC) cells, become addicted to glutamine. Thus, targeting glutamine metabolism emerges as a promising strategy in cancer therapy, with several drugs currently undergoing clinical investigations. Among these, CB-839 is a potent and selective inhibitor of glutaminase-1, the rate-limiting enzyme of glutaminolysis. Although CB-839 is already in clinical trials, a thorough evaluation of its antitumor activity in CRC cells remains critical. This study aimed to explore the antitumoral potential and the metabolic effects of CB-839 on CRC cells.

METHODS

Three CRC cell lines (HCT116, HT29, SW480) were exposed to different CB-839 concentrations (2.5-20 μM). CB-839 cytotoxic effects were assessed by MTT assay. Cell cycle progression was evaluated using flow cytometry analysis. Metabolomic phenotype and ATP production rates were explored via Seahorse Agilent analysis. Metabolomic profile was evaluated with untargeted GC-MS and 1H-NMR, complemented by targeted GC-MS/MS analysis of the Krebs cycle. Finally, data underwent multivariate and univariate statistical approaches (SIMCA16, Umetrics).

RESULTS

CB-839 exerted cytotoxic effects in HT29 cells (CC50: 8.75 μM at 96h). HCT116 and SW480 cells were less sensitive. Following experiments focused on the most and the least respondent CRC cell (HT29 and SW480, respectively). CB-839 determined S-phase accumulation and concomitant decrease of cells in G2/M-phase in HT29 cells, while no alterations were observed in SW480 cell cycle progression. Unlike SW480, HT29 cells exploited more glycolysis than cellular respiration to produce ATP after CB-839 treatment. Metabolomics revealed profound alterations in both investigated cell lines, especially in Krebs cycle and glutaminolysis, along with alterations in aminoacidic (as alanine, aspartate, glycine) sugar (glucose and fructose), and antioxidant (GSH) contents.

CONCLUSIONS

This study confirmed the crucial role of glutamine metabolism in CRC cells, offering insights into the characteristics and mechanisms that determine their sensitivity or resistance to CB-839, thereby providing a strong rationale for promising combined therapy.

P091

EXPLORING SORTILIN AS A POTENTIAL TERAPEUTIC TARGET FOR HYPERTENSION-RELATED COGNITIVE IMPAIRMENT: INSIGHTS FROM PRECLINICAL AND HUMAN STUDIES

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BACKGROUND-AIM

Arterial hypertension affects a notable portion of the elderly population and can be considered an important risk factor for vascular cognitive impairment and dementia, as it contributes to cerebromicrovascular endothelial dysfunction and neurovascular uncoupling. Additionally, hypertension plays a major role in the development of carotid artery stenosis (CAS), which further leads to cognitive impairment. However, the mechanisms underlying these effects remain poorly understood. Recent studies have highlighted sortilin's implication in cardiovascular disease and dementia development. Therefore, we aimed to assess the potential role of sortilin in the molecular mechanism linking hypertension and cognitive impairment.

METHODS

We conducted immunohistochemical analysis on C57BL/6J mice brain implanted with osmotic minipumps releasing recombinant sortilin protein or vehicle and monitored for blood pressure (BP) levels. In vitro studies were performed on Human Brain Microvascular Endothelial Cells (HBMECs) treated with Angiotensin II (Ang II) and pre-transfected with siRNA against sortilin. We also enrolled and collected specimens from 53 hypertensive patients with CAS, stratified for disease severity, who underwent endarterectomy surgery at the "San Giovanni di Dio e Ruggi d'Aragona" University Hospital of Salerno. Gene expression analysis was performed on surgically explanted carotid plaques.

RESULTS

Compared to the vehicle group, chronic sortilin infusion in mice resulted in increased arterial BP, as well as elevated brain expression of the glial fibrillary acidic protein (GFAP) marker of astrogliosis, and higher brain expression of 3-nitrotyrosine (3-NT) and 4-hydroxynonenal (4-HNE) markers of lipid peroxidation. In addition, in HBMECs, sortilin silencing prevents AngII-induced reduction of vascular endothelial-derived adhesion protein (VE-cadherin), the major adhesion protein that controls vascular integrity. Moreover, expression of SORT mRNA was elevated in the carotid plaque from severe CAS patients compared to the mild group, and OSTEOCALCIN mRNA levels paralleled this increase.

CONCLUSIONS

Our data suggest that sortilin could play a key role in hypertension-induced cognitive impairment, highlighting its potential as a novel therapeutic target.

P092

OSM/OSMRβ AXIS CAN SHAPE TUMOR MICROENVIRONMENT AND FAVOUR MASH-RELATED HEPATOCELLULAR CARCINOMA IMMUNE EVASION

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BACKGROUND-AIM

Oncostatin M (OSM), an IL-6 family cytokine, promotes the progression of MASH to HCC through autocrine/paracrine signaling via its receptor OSMRβ, expressed by tumor cells. This study explores the role of the OSM/OSMRβ axis in driving an immunosuppressive tumor microenvironment in MASH-related HCC.

METHODS

OSM role was investigated through combined analyses of samples from cohorts of MASLD/MASH patients with or without HCC, MASH-related HCCs originating in wild type and hepatocyte-specific OSM receptor-β (hOSMRβ-/-) deficient mice and in vitro experiments performed on liver cancer and immune cell lines.

RESULTS

Analysis of OSM-expressing HCC patients with mixed etiology (TCGA-database) revealed strong positive correlations between OSM transcripts and multiple tumor immune microenvironment (TIME) markers. A similar transcriptomic signature was also observed in murine MASH-HCC tumors. Genetic deletion of hOSMRβ in mice significantly reduced tumor volume and weight without affecting macrophage infiltration and OSM production. However, TIME-related gene expression was notably diminished in hOSMRβ-/- tumors, alongside reduced STAT3 phosphorylation and COX-2 activity. Blocking autocrine OSM signaling in HepG2 cells overexpressing OSM prevented TIME marker induction in co-cultured macrophage-derived THP1 cells. This potential role for OSM/OSMRβ in modulating TIME is further supported by the development of tertiary lymphoid structures observed in hOSMRβ-/- vs WT mice. Single cell transcriptomics on human HCCs identified malignant hepatocytes as source of CCL15, a cytokine linked with immunosuppression in HCCs. Circulating CCL15 levels were significantly elevated in MASH-HCC patients as compared to other aetiologies and its expression was also upregulated in experimental MASH-related HCC, correlating with OSM expression and markedly reduced by hOSMRβ deletion.

CONCLUSIONS

The OSM/OSMRβ axis fosters an immunosuppressive TIME in MASH-related HCC by promoting cytokine networks such as CCL15, leading to TAM recruitment and T cell exhaustion. Targeting this pathway may reprogram the tumor immune landscape, offering therapeutic potential in this high-risk patient population.

P093

CLEAVED AND UNCLEAVED FORMS OF SHIGA TOXIN 2 IN THE PATHOGENESIS OF HEMOLYTIC UREMIC SYNDROME

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BACKGROUND-AIM

Hemolytic uremic syndrome (HUS) is a severe life-threatening consequence of Shiga toxin 2 (Stx2)-producing Escherichia coli (STEC) intestinal infections, clinically characterized by thrombocytopenia, hemolytic anemia and acute renal failure.
Once released in the bloodstream of STEC-infected patients, Stx2 may circulate in two forms: uncleaved (with an intact A chain) and cleaved (where the A subunit is split into two fragments connected by a disulfide bridge). Although both forms are toxic to target cells, they bind very differently to human circulating cells and blood components, hence they may differently influence the subsequent phases of HUS pathogenesis, such as the formation of blood cell-derived pathogenic extracellular vesicles (EV) containing Stx2, which are considered the trigger for HUS development.

METHODS

Human blood was challenged with 2 nM of uncleaved or cleaved Stx2 and EV were isolated by differential centrifugation, their number and size determined by nanoparticle tracking analysis and their characterization performed by capillary Western blotting. Vero cells were treated with different concentrations of isolated EV or with free uncleaved or cleaved Stx2 and cell viability was assessed.

RESULTS

Uncleaved Stx2 stimulated the release of a greater number of EV (2.02×10sup11/ml) compared to cleaved Stx2 (1.59×10sup11/ml) or controls (1.31×10sup11/ml) and particularly of the larger EV components (>300 nm diameter).
Uncleaved Stx2-induced EV showed an increased expression of Alix (175.5% ± 41.1, $p<0.05$), as EV marker, and of platelet antigen CD42a (188.0% ± 16.9, $p<0.001$) and leukocyte antigen CD45 (155.0% ± 44.5, $p=0.099$) compared to control EV. When the expression due to uncleaved Stx2 was set at 100%, the stimulation induced by cleaved Stx2 appeared significantly lower: Alix (16.8% ± 27.8, $p<0.01$), CD42a (19.6% ± 34.0, $p<0.05$) and CD45 (4.2% ± 7.4, $p<0.0001$). Uncleaved Stx2-induced EV (3 µl) were more toxic to Vero cells than cleaved Stx2-induced EV (%viable cells 53.5 % ± 1.6 vs 73.8 % ± 9.4; $p<0.05$), although the two forms of Stx2 were equally toxic to the same cells (IC50=0.232 pM and 0.202 pM).

CONCLUSIONS

These findings suggest that cleaved Stx2 by inducing the formation of lower amounts of pathogenic EV in patients' blood, differently influence the course of STEC infections.

P094

AFUCOSYLATED IGG AS A POTENTIAL BIOMARKER FOR INFLAMMATION-DRIVEN DISORDERS: A LECTIN-BASED SCREENING STRATEGY

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BACKGROUND-AIM

The interaction between the Fc region of IgG antibodies and Fc gamma receptors (FcγRs) is regulated by Fc glycosylation. Specifically, specific monosaccharides (e.g., fucose, galactose, sialic acid) influence IgG affinity for FcγRs and shape its effector function toward either pro- or anti-inflammatory outcomes. In COVID-19, a pro-inflammatory IgG glycosylation profile has been associated with severe disease. Notably, anti-spike IgG antibodies lacking fucose residues (afucosylated IgG, af-IgG) exhibited increased affinity for FcγRIIIa enhancing antibody-dependent cellular cytotoxicity (ADCC), as well as for FcγRIIa enhancing platelet activation. Thus, aberrant glycosylation is likely associated to severe COVID-19 symptoms. The af-IgG profile has also been linked to other inflammation-driven conditions, such as Dengue fever, HIV, and alloimmune disorders.
In this study, IgG glycosylation was investigated in a patient suffering from vaccine-induced immune thrombotic thrombocytopenia (VITT), a severe inflammatory complication following ChAdOx1 nCoV-19 (AstraZeneca) vaccination. A specific method to detect af-IgG has been set up.

METHODS

Total IgGs were purified from plasma of 1 VITT patient and 4 vaccinated controls (2 ChAdOx1 and 2 Comirnaty, Pfizer–BioNTech) and analyzed by mass spectrometry (MS). Aleuria aurantia lectin (AAL), which specifically binds α1-6 fucose residues, has been used in a Western blotting assay to detect fucose on purified IgGs.

RESULTS

MS analysis showed that IgGs from the VITT sample exhibited a higher abundance of af-IgG compared to all controls. The same ChAdOx1-vaccinated individuals analyzed by MS, along with additional ChAdOx1-vaccinated plasma samples, were also tested. The relative af-IgG levels detected by AAL blotting reflected the trends observed by MS. Furthermore, inter-individual variability in af-IgG levels was observed.

CONCLUSIONS

MS data support the involvement of af-IgGs in driving severe inflammatory-related symptoms, suggesting the potential utility of an accessible screening method for af-IgG detection. AAL Western blotting has proven to be suitable screening tool to identify individuals with elevated af-IgG, potentially serving as a risk stratification biomarker for vaccine-related or other inflammation-based complications.

P095

IGF1 INDUCES AGGRESSIVE FEATURES THROUGH IL-1β IN TRIPLE-NEGATIVE BREAST CANCER CELLS (TNBC)

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BACKGROUND-AIM

Obesity is an acknowledged risk factor for breast cancer (BC). A relationship between obesity and triple-negative breast cancer (TNBC) subtype has been reported, even though the underlying mechanisms remain to be disclosed. High circulating levels of the insulin-like growth factor 1 (IGF1) have been shown to play a role in the risk of BC development in obese women, mainly through the activation of its cognate receptor IGF1R. In this context, the characterization of the molecular routes by which IGF1 triggers aggressive features in TNBC cells may offer promising avenues for novel therapeutic options in obese TNBC patients characterized by elevated IGF1 levels.

METHODS

Gene expression and Chromatin Immunoprecipitation experiments, ELISA, immunoblotting, immunoprecipitation combined with two-dimensional and three-dimensional in vitro model-based studies, were used to investigate the molecular events through which IGF1 may promote proliferative and motile responses in TNBC cells. Bioinformatics analyses, which have been performed using extensive TNBC patient databases, supported the translational relevance of the results obtained.

RESULTS

We assessed that the IGF1/IGFIR axis induces the collagen VI-dependent activation of the discoidin domain receptor 1 (DDR1) and the subsequent u-regulation of the G protein estrogen receptor (GPER), toward an increased expression and secretion of interleukin (IL)-1β in TNBC cells. As a biological consequence, the IL-1β/IL1R1 signaling promotes in an autocrine manner proliferative and invasive phenotypes in TNBC cells.

CONCLUSIONS

Our results show that the IGF1R/DDR1/GPER transduction signaling triggers IL-1β induction and secretion upon IGF1 exposure in TNBC cells, suggesting that IL-1β/IL1R1 axis may be considered as a therapeutic target in more comprehensive approaches in obese TNBC patients exhibiting high IGF-1 bioavailability.

P096

EPIGENETIC SIGNATURES OF TAMOXIFEN RESISTANCE: TOWARD PREDICTIVE BIOMARKERS IN ER+ BREAST CANCER

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BACKGROUND-AIM

Breast cancer is the most frequently diagnosed cancer among women, with a large proportion of patients having estrogen receptor–positive (ER+) tumors, which generally respond to endocrine therapies like tamoxifen. However, primary or acquired resistance to these therapies remains a major clinical challenge. Although the molecular mechanisms of tamoxifen resistance are multifactorial, the role of DNA methylation is poorly understood. To address this, we performed an integrative methylome and transcriptome analysis of tamoxifen-sensitive and -resistant breast cancer cell line models.

METHODS

DNA methylation and hydroxymethylation levels were assessed in six pairs of tamoxifen-sensitive and -resistant cell lines (MCF7, MCF7L, T47D, T47D S2/TR1, ZR75.1, and BT474) using colorimetric ELISA and Infinium HD Methylation assays. Additionally, RNA-seq was conducted for the MCF7 cell line pair.

RESULTS

Global methylation and hydroxymethylation analyses revealed marked epigenetic differences between tamoxifen-resistant and sensitive cell lines. Microarray-based methylation profiling showed that 98% of differentially methylated CpGs (DMCs) were hypermethylated in the resistant lines, with 14.8% located in promoter regions. To correlate DNA methylation with gene expression, we focused on promoter-associated DMCs and performed RNA sequencing on MCF7 cell lines, identifying 1,494 hypermethylated downregulated and 923 hypomethylated upregulated genes. A robust signature of 395 genes showed consistent methylation patterns in at least four out of the six resistant versus sensitive cell lines. Enrichment analysis revealed key pathways associated with tamoxifen resistance, including early and late estrogen response, epithelial-mesenchymal transition, and hypoxia. Furthermore, we applied a Mixomics integrative model capable of distinguishing tamoxifen-resistant from sensitive cell lines, identifying a panel of 11 CpGs with strong discrimination power.

CONCLUSIONS

Our findings highlight the role of DNA methylation in mediating tamoxifen resistance in breast cancer and identify potential biomarkers with predictive and therapeutic relevance. Validation in patient cohorts will be critical to translating these findings into clinical tools for precision oncology.

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P097

GLUTATHIONE-COATED GOLD NANOPARTICLES AS α-KETOGLUTARATE CARRIERS: A NOVEL THERAPEUTIC STRATEGY FOR LUNG ADENOCARCINOMA

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BACKGROUND-AIM

Lung adenocarcinoma (LUAD) remains one of the deadliest cancers worldwide. Altered glucose metabolism and dysregulated epigenetic mechanisms are key hallmarks of LUAD progression. Alpha-ketoglutarate (α-KG), a tricarboxylic acid cycle intermediate, plays a role in both metabolism regulation and histone demethylation. However, its clinical application is limited by poor stability and low cellular uptake. Gold nanoparticles (AuNPs), especially when coated with glutathione (GSH), represent a promising delivery system due to their stability, biocompatibility, and potential for functionalization.

METHODS

GSH-AuNPs were synthesized via chemical reduction and characterized using Nanoparticle Tracking Analysis (NTA), Nuclear Magnetic Resonance (NMR) spectroscopy, and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Binding interactions between AuNPs and α-KG were evaluated through NMR and EXSY spectroscopy. A549 LUAD cells were cultured under varying glucose conditions and treated with either free or nanoparticle-bound α-KG. Cellular uptake, proliferation, and transcriptomic changes (RNA-seq) were analyzed. In vivo biodistribution was assessed following intratracheal administration of GSH-AuNPs in mice.

RESULTS

GSH-AuNPs exhibited a narrow size distribution (~130 nm) and high colloidal stability. NMR confirmed reversible binding of α-KG to the nanoparticle surface. GSH-coated gold nanoparticles demonstrated no cytotoxicity in MCF7, PEO4, and A549 cell lines, maintaining cell viability across all tested concentrations. NMR spectroscopy confirmed that α-KG efficiently and reversibly binds to the nanoparticle surface, indicating stable yet dynamic interactions. Transcriptomic analysis revealed that α-KG delivered via GSH-coated nanoparticles was able to induce distinct gene expression changes. Biodistribution analysis confirmed nanoparticle accumulation in lung tissue.

CONCLUSIONS

GSH-coated AuNPs effectively deliver α-KG to LUAD cells, enhancing its stability and cellular activity. This nanoparticle-based strategy modulates both metabolic and epigenetic pathways, offering a novel promising therapeutic approach in LUAD. Further preclinical studies are needed to validate its translational potential.

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P098

PHOSPHOLIPID AND NON-PHOSPHOLIPID VESICLES FOR PHENFORMIN SELECTIVE TARGETING IN SHH MEDULLOBLASTOMA

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common pediatric brain tumor, often associated with activating mutations in the Sonic Hedgehog (SHH) pathway. Previously, we demonstrated that the antidiabetic drug phenformin (Phen) significantly inhibits the SHH-dependent transcription factor Gli1 in preclinical models of SHH-driven medulloblastoma (SHH MB), resulting in substantial tumor growth reduction. However, Phen is associated with an elevated risk of lactic acidosis. Therefore, delivering Phen through carriers that bypass the liver and specifically target the brain could be a valuable strategy.

METHODS

To decrease carrier-induced toxicity and increase plasma half-life, phenformin-loaded niosomes (Ph-NVs) were created using the film approach. To assess the in vitro cytotoxicity, Med1-MB cells were exposed to progressively higher concentrations of Ph-NVs and cell viability was evaluated. Cells were treated, collected, and analyzed with a fluorescent microscope to investigate cellular uptake. Ph-NVs were administered to mice to examine the in vivo pharmacokinetic distribution. After injection, tissue and plasma samples were collected and phenformin measured. Additionally, qRT-PCR was performed in cultured cells and tumor samples from mouse models.

RESULTS

In this study, we show that loading phenformin into specific liposome-like delivery systems (niosomes) efficiently leads to its release to SHH medulloblastoma tumors. We demonstrate that Ph-NVs are incorporated into Med1-MB SHH medulloblastoma cells, where they significantly reduce cell proliferation. Phenformin-loaded nanocarriers decrease SHH signaling, as evidenced by a marked reduction in the expression of the SHH target genes Gli1 and Ptch1. Remarkably, when phenformin-loaded vesicles were administered to mouse models of SHH MB, Phen concentrations were substantially reduced in the liver and plasma, while significantly increased in cerebellar tumors compared to the drug alone. Correspondingly, intratumor levels of Gli1 and Ptch1 mRNAs were markedly downregulated, further validating this approach for targeting SHH signaling in vivo.

CONCLUSIONS

Overall, these findings illustrate a novel nanodelivery-based approach to inhibit oncogenic Hedgehog signaling and enhance the biodistribution of phenformin, potentially reducing its side effects.

P099

COMPUTATIONAL-AIDED DISCOVERY OF A NOVEL MYC INHIBITOR WITH EPIGENETIC IMPLICATION IN ANTICANCER ACTIVITY

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BACKGROUND-AIM

Myc is a transcription factor whose dysregulation contributes to cancer onset and progression. Beyond promoting proliferation and survival, Myc acts as an epigenetic amplifier by recruiting chromatin modifiers and reshaping the transcriptional landscape. However, its intrinsically disordered structure complicates direct targeting by conventional drug design. The aim of this study was to identify a small molecule capable of modulating Myc's oncogenic activity, particularly its epigenetic and transcriptional functions, to develop new therapeutic strategies.

METHODS

A structure-based virtual screening of over 183,000 compounds was conducted to identify potential Myc inhibitors. Surface Plasmon Resonance (SPR) was employed to validate binding to Myc and its partner Max. Functional effects were assessed in leukemia (U937) and colorectal cancer (HCT-116 and HT-29) cell lines, along with normal keratinocytes, by FACS analysis. Protein structural changes were analyzed via targeted limited proteolysis (t-LiP) proteomics. Transcriptional effects, by Western Blot analysis, including p21 regulation, were evaluated to assess downstream Myc target modulation.

RESULTS

The screening led to the identification of S19, a novel small molecule that binds directly to both Myc and Max, disrupting their oncogenic heterodimer. S19 selectively reduced Myc protein levels, induced G2/M cell cycle arrest, and had no significant effects on normal keratinocytes. t-LiP analysis revealed structural alterations in Myc upon S19 treatment. Moreover, S19 upregulated p21 expression, indicating a disruption of Myc-dependent repression and alteration of chromatin-associated transcriptional regulation.

CONCLUSIONS

S19 is a promising small molecule that targets Myc and its transcriptional network, exerting antiproliferative effects through modulation of gene expression and epigenetic control. These findings highlight the potential of S19 as a therapeutic scaffold for developing novel cancer treatments targeting Myc-driven pathways.

P100

ASSESSMENT OF INTERLEUKIN-1β ACTION IN TRIPLE-NEGATIVE BREAST CANCER (TNBC) MICROENVIRONMENT

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BACKGROUND-AIM

Cancer-associated fibroblasts (CAFs), which are main players within the breast cancer (BC) stroma, trigger multilayered communications with cancer cells through the secretion of various types of molecules. In this context, interleukin (IL)-1β has emerged as a crucial mediator of tumor-promoting inflammatory responses within the tumor microenvironment (TME). The contribution of a pro-inflammatory TME to BC progression, even in the aggressive subtypes named triple-negative breast cancer (TNBC), has been largely acknowledged like in obese women. Elucidating the molecular mechanisms by which an inflammatory TME fosters cancer progression and dissemination still represents a major challenge for the development of novel therapeutic strategies in TNBC patients.

METHODS

Gene expression and immunofluorescence studies, combined with 3D growth and migration assays, were performed to ascertain whether inflammatory molecules deriving from TNBC cells may mediate the activation of normal fibroblasts into CAFs-like cells, toward the promotion of aggressive features within the TNBC microenvironment.

RESULTS

IL-1β signaling has been found to mediate the reprogramming of normal fibroblasts into CAFs. In particular, CAFs-like cells displayed higher protein levels of a distinctive CAFs marker named FAP respect to the normal counterpart. Moreover, in line with a paracrine functional interaction typically occurring between cancer and stromal cells, we demonstrated that activated fibroblasts contribute to boosting the growth and migration behaviour of TNBC cells.

CONCLUSIONS

Our findings highlight the role of IL-1β in promoting a TME that facilitates TNBC progression. Importantly, our study underscores the importance of patient stratification for designing personalized therapeutic strategies targeting tumor-stroma interactions in TNBC patients characterized by an inflammatory TME.

P101

MELANOMA IN DISGUISE: HOW ANDROGEN RECEPTOR ACTIVATION DRIVES IMMUNE ESCAPE AND TUMOR INVASIVENESS

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BACKGROUND-AIM

Malignant melanoma is the fifth most frequent cancer worldwide, with a steadily increasing incidence. Although targeted therapies and immune checkpoint inhibitors have significantly improved patient survival, resistance and disease progression remain major clinical challenges. Interestingly, gender disparities in outcomes have been observed, with females showing a survival advantage. Recent evidence suggests that the androgen/androgen receptor (AR) axis may promote melanoma progression, yet the underlying mechanisms remain largely unexplored.
We hypothesized that androgen receptor signaling contributes to immune escape in melanoma by interfering with natural killer (NK) cell-mediated cytotoxicity. This study aimed to elucidate the molecular mechanisms by which AR influences melanoma immune evasion and to evaluate its clinical relevance.

METHODS

We employed a combination of cell-based assays, co-cultures with primary NK cells, pharmacological inhibition, siRNA approaches, 3D models and protein analysis to assess the impact of AR activation on immune sensitivity. In particular, we focused on the regulation of MICA, a key stress ligand for the NK cell receptor NKG2D. Serum levels of soluble MICA (sMICA) were evaluated in melanoma patients undergoing anti-PD-1 immunotherapy (pembrolizumab), and in vitro responsiveness to checkpoint blockade was assessed following AR depletion.

RESULTS

Activation of the androgen receptor enhanced melanoma invasiveness and significantly impaired NK cell-mediated cytotoxicity. Mechanistically, this was linked to increased shedding of surface MICA, mediated by AR-dependent upregulation of the metalloprotease ADAM10. Pharmacological blockade of AR or ADAM10 efficiently prevented MICA loss and restored NK cell recognition. Clinically, high sMICA levels in the serum correlated with poor outcome in melanoma patients treated with the anti-PD-1 monoclonal antibody. Importantly, AR-deficient melanoma cells displayed increased susceptibility to immune checkpoint inhibitors.

CONCLUSIONS

Our findings reveal a novel role for androgen receptor signaling in melanoma immune escape via modulation of MICA shedding. These results support the use of AR as a biomarker of immune resistance and suggest that AR antagonists may enhance the efficacy of immunotherapies in melanoma patients.

P102

ANTITUMOR EFFECTS OF THE AXL INHIBITOR TP-0903 ON MALIGNANT MESOTHELIOMA

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BACKGROUND-AIM

Malignant Mesothelioma (MM), a rare tumor arising from the mesothelial cells that line the serosal cavities, is characterized by a very poor prognosis. Indeed, current treatment options have limited efficacy on this tumor and research is increasingly focusing on new targeted therapy approaches. In this study, the antitumor effects of TP-0903, an inhibitor of the tyrosine kinase receptor AXL, were analyzed on different human MM cell lines reflecting the three different MM histotypes: epithelioid, sarcomatoid, and biphasic.

METHODS

The effects of TP-0903 on cell proliferation, death, migration and on the modulation of signaling pathways deregulated in MM were evaluated in cultured MM cell lines by Sulforhodamine B assay, FACS analysis, Trypan Blue exclusion, Wound Healing assay and Western blotting. Transcriptomic analysis was performed using nCounter Tumor Signaling 360 panel. The effects of TP-0903 were also evaluated in tumor spheroids, generated by plating cells in 96-well round-bottom ultra-low adherent plates. Spheroid responses to drug treatments were evaluated in terms of changes in three-dimensional growth (by measuring spheroid diameter), viability (by RT-GLO assay), and apoptosis (by caspase-3/7 Glo assay and Annexin V/PI assay).

RESULTS

TP-0903 was able to inhibit MM cell growth in a time- and dose-dependent manner, both in 2D and 3D cell culture conditions. The sarcomatoid and biphasic MM cell lines were the most sensitive to TP-0903 treatment. TP-0903 induced apoptosis in all the cell lines analyzed, both in 2D and 3D conditions. TP-0903 inhibited cell migration in a dose-dependent manner. Western blotting analysis demonstrated modulation of Axl, ErbB receptors, MAPKs and AKT expression and activation after TP-0903 treatment. Lastly, transcriptomic analysis highlighted the presence of differentially expressed genes in the treated cells, specifically related to cell cycle, migration, proliferation and death.

CONCLUSIONS

This study demonstrates the antitumor potential of TP-0903 on MM by using 2D cell cultures and 3D cell spheroids, which are known to provide a more clinically realistic model of tumor behavior compared to 2D cultures. Overall, our findings could have important implications for the future development of TP-0903-based therapies for MM patients.

P103

TARGETING BRPF1/ASH1L CO-DEPENDENCY SYNERGISTICALLY COUNTERACTS ENDOCRINE THERAPY RESISTANCE IN BREAST CANCER.

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BACKGROUND-AIM

Endocrine therapy (ET) resistance affects ~30% of ERα-positive (ERα+) breast cancers (BCs), often due to aberrant epigenetic regulation of estrogen signaling. Epigenetic enzymes and chromatin remodelers, co-acting with ERα, contribute to tumor progression and therapy failure. Thus, dissecting these co-dependencies may reveal actionable targets to counteract resistance mechanisms.

METHODS

Using DepMap co-essentiality mapping, we identified genetic dependencies of Bromodomain and PHD Finger Containing 1 (BRPF1), an essential epigenetic regulator in ERα+ BC. We validated our findings via pharmacologic inhibition and in vitro/ in vivo functional assays in both ET-sensitive and -resistant BC models. Additional computational and molecular analyses were performed to assess gene interaction and pathway alterations.

RESULTS

BRPF1 co-dependency mapping revealed ASH1L, a histone methyltransferase of the Trithorax complex, as a novel partner. ASH1L blockade caused marked dysregulation of estrogen signaling, increased apoptosis, and defects in DNA damage response. Moreover, concomitant inhibition of BRPF1 and ASH1L induced a synergistic effect in overcoming ET resistance.

CONCLUSIONS

We revealed a novel BRPF1/ASH1L epigenetic axis crucial for BC cell survival and proliferation and targeting this co-dependency represents a promising therapeutic strategy to treat aggressive ET-resistant ERα+ BCs.

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P104

TARGETING TUMOR GROWTH WITH A NEW ERK5 KINASE INHIBITOR

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BACKGROUND-AIM

The Mitogen-Activated Protein Kinase (MAPK) Extracellular-signal Regulated Kinase 5 (ERK5) pathway is involved in most cancer hallmarks, with a well-established role in tumor development and progression. ERK5 activation is also a known resistance mechanism to RAS/RAF/MEK1/2-ERK1/2 targeted therapies. Although several ERK5 kinase inhibitors have been developed, they often require high concentrations and may paradoxically activate ERK5 by promoting its nuclear translocation and the subsequent transcriptional activation of target genes, limiting their suitability for clinical application. Therefore, we aimed to develop new ERK5 kinase inhibitors.

METHODS

To test compound efficacy on ERK5 kinase activity, we performed in vitro kinase assays. Cytotoxic effects were evaluated via trypan blue exclusion, colony formation, and spheroid growth assays using wild-type and ERK5-knockout (KO) cells.

RESULTS

In silico screening of the MolPort commercial database and of the National Cancer Institute led to the identification of 12 candidate compounds. In vitro kinase assays pinpointed one compound (compound 2) inhibiting ERK5 kinase activity from 100 nM, with full inhibition at 600 nM. Notably, compared to the commercial inhibitor JWG-071, compound 2 showed efficacy at lower doses. Proliferation assays in melanoma, ovarian, and hepatocellular carcinoma cell lines showed dose-dependent inhibition by compound 2. Interestingly, performing the same experiments using control and ERK5-KO cells, both in 2D and 3D models, resulted in a decreased sensitivity to compound 2 in ERK5-KO cells with respect to the control, highlighting an on-target effect. When comparing these findings with those for JWG-071, we observed that compound 2 exhibits higher specificity in inhibiting cell proliferation. Similar results were obtained in colony formation assays.

CONCLUSIONS

We identified a new ERK5 kinase inhibitor that works at nanomolar concentrations, effectively inhibiting both ERK5 kinase activity and cell proliferation in an ERK5-dependent manner. Although further studies are necessary to confirm that this inhibitor does not trigger paradoxical ERK5 activation, these results pave the way to a new class of small molecules targeting ERK5, which could achieve clinical relevance following additional preclinical evaluation.

P105

BRPF1 IN CANCER: PROGNOSTIC ROLE AND THERAPEUTIC POTENTIAL

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BACKGROUND-AIM

BRPF1 is a key scaffold protein in histone acetyltransferase complexes that regulate chromatin remodeling and transcription. Increasing evidence implicates BRPF1 dysregulation in cancer development and progression, potentially through interactions with oncogenic pathways and tumor suppressors, such as mutant p53. This study aimed to investigate BRPF1's prognostic value across different cancer types, its relationship with TP53 mutation status, and the functional impact of BRPF1 inhibition in ovarian cancer (OC), a malignancy with a high frequency of TP53 mutations.

METHODS

BRPF1 expression and prognostic significance were assessed using UALCAN and CSIOVDB databases. CRISPR-Cas9 loss-of-function screens were used to evaluate BRPF1 essentiality in metastatic OC. Functional assays, including wound healing, colony formation, migration, and invasion, were performed to study the effects of BRPF1 silencing or pharmacological inhibition. Cell proliferation, cell cycle progression, and apoptosis were analyzed via MTT, FACS, Caspase 3/7 assays, and Western blotting. RNA-Seq was conducted to identify BRPF1-regulated signaling pathways.

RESULTS

BRPF1 mRNA and protein levels were elevated in multiple tumor types, including gastrointestinal, head and neck squamous cell carcinoma, bladder urothelial carcinoma, glioblastoma, lung squamous cell carcinoma, and OC. BRPF1 expression was significantly higher in TP53-mutant tumors and correlated with poor prognosis in adrenocortical carcinoma, liver hepatocellular carcinoma, mesothelioma, prostate adenocarcinoma, and OC. BRPF1 was found to be essential for metastatic OC cell viability, with its expression increasing with tumor stage, grade, and metastatic potential. Pharmacological inhibition using GSK6853 or its analog GSK5959 reduced OC cell proliferation, impaired migratory and invasive abilities, and induced cell cycle arrest, apoptosis, and DNA damage - effects that were also observed with BRPF1 silencing. RNA-Seq analysis revealed that BRPF1's antiproliferative effects may be mediated by PPARα pathway modulation.

CONCLUSIONS

BRPF1 represents a potential prognostic marker and a promising therapeutic target across several cancer types, particularly in metastatic OC.

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P106

A PH-RESPONSIVE MESOPOROUS SILICA NANOSYSTEM CONJUGATED WITH FOLIC ACID FOR SELECTIVE DOXORUBICIN DELIVERY

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BACKGROUND-AIM

Cancer is a significant global health issue, responsible for nearly 20% of deaths in 2021. One of the major challenges in cancer treatment is the development of therapeutic strategies that selectively target tumor cells while minimizing harm to normal tissues. Localized drug delivery to carry the chemotherapy drug doxorubicin (DOXO), was developed. This device, referred to as FOL-MSN-DOXO, releases the drug in response to the acidic environment of tumors and incorporates folic acid (FOL) for targeting purposes. The effectiveness of FOL-MSN-DOXO was tested in vitro against folate receptor-expressing (FR+) cancer cells, as well as against folate receptor-negative (FR-) normal (healthy) cells.

METHODS

The endocytosis of mesoporous silica nanoparticles (MSNs) was monitored through transmission electron microscopy (TEM). The efficacy of FOL-MSN-DOXO was evaluated using growth experiments, spheroid formation assay, Annexin V and reactive oxygen species (ROS) measurements, Western Blot analysis, and immunostaining on rat Dorsal Root Ganglion (DRG) cells.

RESULTS

FOL-MSN-DOXO effectively killed FR+ cancer cells while sparing FR- normal cells. In contrast, free DOXO was toxic to all cell lines, regardless of FR expression. The uptake of MSNs occurred exclusively in FR+ cells via FR- mediated endocytosis, with no uptake observed in FR- cells. Both FOL-MSN-DOXO and free DOXO significantly increased ROS production, leading to apoptosis. However, only free DOXO induced ROS and triggered apoptosis in FR- normal cells. Importantly, the vehicle alone (FOL-MSN) showed no toxicity in any tested cell lines, and immunostaining on DRG cells demonstrated significantly lower neuronal toxicity for FOL-MSN-DOXO compared to free DOXO.

CONCLUSIONS

The FOL-MSN-DOXO nanosystem specifically targets cancer cells that express FR, offering enhanced safety and comparable effectiveness against tumors when compared to conventional doxorubicin formulations. As a result, it presents a promising strategy for the targeted and safe delivery of chemotherapy in the treatment of cancers that express folate receptors.

P107

EXPLORING THE IMMUNE CONTEXTURE OF HER2+ AND TRIPLE-NEGATIVE BREAST CANCER BRAIN METASTASES

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BACKGROUND-AIM

Breast cancer brain metastases (BCBM) are a significant challenge in breast cancer, associated with poor prognosis. The tumour microenvironment (TME) of BCBM is heterogeneous and remains poorly characterized, particularly regarding subtype-specific differences and their prognostic implications. Understanding the TME at metastatic site is essential to identify key immune cell populations and their spatial organization in the brain metastatic niche, ultimately aiming to uncover potential therapeutic targets.

METHODS

In this study, we examined formalin-fixed, paraffin-embedded (FFPE) brain metastasis tissue sample from 14 HER2 positive and 12 triple-negative breast cancer (TNBC) patients. We performed spatial phenotyping by Tyramide Signal Amplification-based Opal multiplex immunofluorescence (mIF) across three panels enabling the characterization of key cell types including T lymphocytes (CD4+, CD8+, CD3+), B cells (CD20+), microglia/macrophages (IBA1+,CD68+), neutrophilsCD66b+, MPO+), tumor associated neutrophils (TANs: CD66b+,IL8+), tumour (PANCK+, CD147+) and endothelial cells (CD31+).

RESULTS

Our results show that the number ofTANs is higher than in the brain metastases (BM) of TNBC patients compared to HER2+ as well asan increased number of CD20+ and CD8+ cells. Spatial analysis revealed that neutrophils are more distant from IL-8 expressing endothelial cells (CD31+IL8+) and macrophages (CD68+) in TNBC compared to HER2+ patients but are more closely located near CD4+ cells. Furthermore, immune cells in TNBC (CD8+, CD4+, CD68+) are found in closer proximity to one another compared to those in HER2+ BM. These findings highlight a contrasting immune and inflammatory landscape within the TME of the two subtypes. In TNBC, the TME may facilitate unique interactions between tumour cells, neutrophils and other immune populations that differ from those observed in HER2+ tumours. This suggests a distinct spatial organization of the immune system and TME in TNBC, which may profoundly influence tumour progression and response to therapy.

CONCLUSIONS

Understanding these spatial immune dynamics provides a promising foundation for translational research, with the potential to guide the development of subtype-specific immunotherapeutic approaches tailored to the distinct biology of metastatic TNBC and HER2+ breast cancer.

P108

CHARACTERIZATION OF STAGE I RETINAL ORGANOIDS: A PROTEOMIC APPROACH

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BACKGROUND-AIM

Glaucoma is a leading cause of irreversible blindness, primarily treated by reducing intraocular pressure (IOP) with topical medications. However, some patients experience disease progression despite reaching target pressure. A limited understanding of the molecular mechanisms behind Retinal Ganglion Cell (RGC) loss hampers the discovery of new druggable targets. Thus, identifying disease-associated genes requires a focus on IOP-independent mechanisms. Recent advancements in stem cell engineering allow the creation of retinal organoids (ROs) for previously unfeasible eye studies. Evidence shows ROs are valuable for pathological and developmental studies, potentially providing retinal cells for vision restoration in glaucoma patients without immune rejection.

METHODS

hIPSCs-derived ROs were obtained using specialized media over time. After differentiation, nanoscale liquid chromatography with tandem mass spectrometry analysis (nLC-MS/MS) was performed for a detailed proteomic analysis of RO-derived cells at T0, T21, T28, T35. Following this, we isolated the CD90+ cell population from ROS at T28 and T35 using low-pressure FACS sorting, as CD90 is uniquely expressed in RGCs in the retina. The resulting populations underwent further analysis with nLC-MS/MS, western blot, and confocal microscopy.

RESULTS

nLC-MS/MS analysis identified 7455 non-redundant proteins. Principal Component Analysis (PCA) showed clear separation of RO-derived cell populations at T35, highlighting the uniqueness of proteins. Gene ontology (GO) analysis in RO at T35 indicated a retinal protein expression pattern involved in neuronal differentiation, synapse organization, neuron development, and eye development. Isolation of positive CD90 cells by low-pressure FACS sorting from ROs at T28 and T35 showed a 15% difference between time points. Our proteomic analysis revealed 6559 proteins, with notable differences between CD90+ and CD90- populations isolated from T35. Additionally, using proteomic data and confocal images, we identified cell phenotypes in the CD90+ subpopulation, including astrocytes, microglia, and RGCs, emphasizing the need for a specific RGC surface marker for better RGC characterization.

CONCLUSIONS

In conclusion, this study provides a robust protein landscape deriving ROs, which will be helpful for future investigations. It also provides a framework for integrating proteomic information for CD90+ cell subtypes.

P109

“FIGHTING MELANOMA DRUG RESISTANCE: SYNERGISTIC EFFECTS OF METFORMIN AND PLX4032 IN PATIENT-DERIVED CELLS”

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BACKGROUND-AIM

Malignant melanoma often harbors the BRAFV600E mutation, rendering it initially responsive to PLX4032 (PLX), a BRAF inhibitor. However, drug resistance commonly arises within six months of treatment. Our previous study demonstrated that PLX resistance of two patient-derived melanoma cell lines is related to oxidative phosphorylation. Therefore, our hypothesis is that metformin—an antidiabetic drug known to inhibit mitochondrial respiration—could represent a promising strategy to overcome resistance in BRAF-mutant melanoma.

METHODS

Experiments were conducted on PLX-resistant MeOV (BRAFV600E) and MeTA (BRAFV600D) metastatic melanoma cells (Garbarino et al., 2022) treated with metformin (10 mM) and/or PLX (1.5 μM) for up to 120 h. Cell responses were assessed through MTT assays (viability/proliferation), X-Gal staining (senescence), LPO staining (lipid peroxidation), and clonogenic assays.

RESULTS

Metformin sensitized both MeTA and MeOV cells to PLX, with a more rapid and marked effect in MeTA cells, which showed a 20% drop in metabolically-active cells within 24 h. Moreover, an induction of cellular senescence and lipid peroxidation was observed in both populations. Noteworthy, metformin alone reduced clonogenic potential in both models while its combination with PLX was able to strongly inhibit clonogenicity only in MeTA cells, indicating a synergistic response.

CONCLUSIONS

These findings suggest that metformin may overcome PLX resistance in BRAF-mutant melanoma. Its ability to induce senescence, reduce viability, and suppress clonogenic growth—especially in MeTA cells—highlights a metabolic vulnerability of resistant metastatic melanoma that could be therapeutically targeted.

P110

MAPPING TREATMENT RESPONSE IN AR+ TRIPLE-NEGATIVE BREAST CANCER USING SPATIAL PROFILING

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BACKGROUND-AIM

Breast cancer is the most common cancer in women globally, with triple-negative breast cancer (TNBC) accounting for 15–20% of cases. TNBC is highly aggressive and lacks targeted therapies. Within this group, the luminal androgen receptor (LAR) subtype, characterized by androgen receptor (AR) expression in 12–50% of cases, is emerging as a distinct clinical entity with therapeutic potential. Although neoadjuvant chemotherapy (NACT) is the standard treatment for early-stage TNBC, many patients with residual disease post-NACT have poor outcomes. The tumor immune microenvironment (TIME) is increasingly recognized as a key factor influencing therapy response, particularly through immune cell composition and spatial dynamics.

METHODS

We analyzed the TIME in AR+ TNBC patients before and after NACT using Tyramide Signal Amplification-based Opal multiplex immunofluorescence (mIF). This spatial phenotyping approach allowed characterization of immune cell populations and their interactions with tumor cells. The panel included markers for CD8, CD4, CD20, Foxp3, TIM3, PD-1, PD-L1, and pan-CK.

RESULTS

Responders to NACT showed higher baseline levels of immune subsets such as CD20#PD-1# B cells, CD4#FOXP3# regulatory T cells, and CD8#PD-1#TIM3# T cells, suggesting a more active and regulated immune environment. Tumor cells (PanCK#) were initially located closer to PD-L1# immunosuppressive cells in responders, but this proximity decreased after NACT, implying reduced immunosuppression. In contrast, non-responders showed increased proximity between tumor cells and immunosuppressive cells post-treatment. Notably, spatial dynamics of B cells and regulatory B cells (Bregs) differed by response: in responders, these cells moved away from tumor areas after NACT, while in non-responders they became more localized near tumor cells, without evidence of effective immune activation.

CONCLUSIONS

These findings support the predictive value of spatial immune profiling in AR+ TNBC and highlight the potential of targeting immune checkpoint pathways to enhance therapeutic response. Further validation in larger patient cohorts is needed to substantiate these observations and explore the functional role of identified immunosuppressive cell populations.

P111

IRF5-RELATED PROINFLAMMATORY ACTIVATION OF MICROGLIAL CELLS IS COUNTERACTED BY NRF2/HO-1

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BACKGROUND-AIM

Microglia are innate immune cells residing in the central nervous system, their dysregulated activation leads to neuroinflammation and neuropathologies through molecular mechanisms not well understood yet. Several studies have highlighted the transcription factor IRF5 as a major player in inducing inflammation through an interaction with the NF-κB pathway. The purpose of our work is to understand how the NRF2/HO-1 axis, an important anti-inflammatory pathway, regulates IRF5 to find new molecular targets to counteract neuroinflammation.

METHODS

Human microglial cell line (HMC3) and murine macrophage-like cells (RAW264.7) were cultured under standard conditions and treated with 100 ng/ml Lipopolysaccharide (LPS), 5 μM Sulforaphane (SFN), 10 μM Tin mesoporphyrin IX (SnMP) and with 20 nM Resiquimod (R848).
HO-1 silencing was performed by using a specific pool of oligonucleotides.
Western Blot analysis was performed to detect proteins of interest in the whole cell lysates and in nuclear fractions.
TNFα release in cell culture medium were detected by using ELISA assay.
mRNA expression was measured by RT-qPCR analysis.
Statistical analysis was performed by applying ANOVA and Tukey's multiple comparison tests.

RESULTS

Microglial cells exposure to SNF, a NRF2 activator, prevented the induction and the release of TNFα and significantly reduced IRF5 nuclear localization in response to LPS stimulation. Notably, SFN reduced IRF5 expression in microglial cells exposed also to R848, used to activate IRF5. Moreover, we observed that TNFα gene expression was higher in cells silenced for HO-1 or exposed to HO-1 inhibitor (SnMP) and exposed to LPS compared to LPS alone. Also, preliminary results showed that HO-1 silencing promotes IRF5 nuclear localization. These findings were confirmed in the murine macrophage-like cell line RAW264.7.

CONCLUSIONS

To conclude, our study demonstrates that the NRF2/HO-1 pathway limits IRF5 activity and reduces microglia's proinflammatory activation. These findings provide new insights into the regulation of neuroinflammation that could inform future therapeutic strategies.

P112

DISCOVERY OF A NEW SELECTIVE ENDOPLASMIC RETICULUM AMINOPEPTIDASE 1 (ERAP1) INHIBITOR FOR HEDGEHOG-DEPENDENT CANCER TREATMENT

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BACKGROUND-AIM

Aberrant activation of the Sonic Hedgehog (SHH) pathway has been associated with the progression of several cancers, including medulloblastoma (MB), one of the most common malignant paediatric brain tumors, which shows a high resistance to current therapeutic approaches. Recently, we identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) as a strong activator of the SHH pathway and promising therapeutic target for the treatment of SHH-MB, however, the lack of selective inhibitors for ERAP1 limits its therapeutic potential. This study aims to discover and characterize a new selective ERAP1 inhibitor and asses its anti-cancer properties to fight SHH-MB growth.

METHODS

N1 was selected by virtual screening of a library of natural compounds against crystallographic structure of the catalytic domain of ERAP1. The efficacy of N1 to inhibit ERAP1 activity was evaluated by an antigen presentation assay in HeLA.Kb and the direct binding between N1 and ERAP1 was confirmed by Cellular Thermal Shift Assay. The ability of N1 to affect SHH pathway activity by perturbing the ERAP1 regulatory axis was assessed by in vitro pull-down and co-IP assays. Finally, the anti-cancer potential of N1 to inhibit SHH-MB growth was investigated in preclinical heterotopic and orthotopic allograft models, including PDX models. The ability of N1 to cross the blood–brain barrier was assessed HPLC/MS analysis of cerebellar tissue from N1-treated mice.

RESULTS

We identified compound N1 as a new selective inhibitor of ERAP1. We found that N1 directly binds ERAP1, altering its interaction with USP7. This event promotes βTrCP protein stability and GLI1 degradation, thereby blocking SHH signalling and SHH-dependent cell proliferation. Importantly, SHH-dependent cell models genetically depleted for ERAP1 are insensitive to N1 treatment, highlighting the specificity of N1 for ERAP1. Notably, we found that N1 crosses the BBB and suppresses SHH-MB growth both in vitro and in vivo.

CONCLUSIONS

Our study reveals the promising anti-cancer properties of N1, making it a good candidate for further preclinical studies and confirming that the pharmacological targeting of ERAP1 is an attractive option for the development of new targeted therapies for the treatment of SHH-dependent tumors.

P113

ANTI-FIBROBLAST ANTIBODIES FROM IGG4-RD PATIENTS MODULATE FIBROBLAST FUNCTION IN VITRO

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BACKGROUND-AIM

IgG4-related disease (IgG4-RD) is a rare fibro-inflammatory condition characterized by tissue fibrosis, a diffuse lymphoplasmacytoid infiltrate, obliterative phlebitis and abundance of IgG4-positive plasmacells. Fibroblast activation and collagen deposition are tightly associated with the presence of IgG4 positive plasmacells in affected tissues and B-cell depletion is paralleled by fibroblast deactivation, suggesting that B cells and fibroblasts may cooperate to sustain tissue fibrosis.
The aim of this project is to evaluate the role of antibodies produced by IgG4-RD B-cells in mediating fibroblast activation.

METHODS

Recombinant monoclonal antibodies (rmAbs) were generated from IgG4-RD patients B cells by FACS sorting, single cell PCR and expression vector cloning. IgVH+VL gene usage, clonal selection and cluster analysis were also performed. Primary human dermal fibroblasts (HDF) were isolated from normal skin. The ability of rmAbs to bind HDF was evaluated by indirect immunofluorescence (IFI) and by western blotting on HDF subcellular extracts. The role of antibodies in modulating cytokine production was evaluated by ELISA and the effect on fibroblast migration was assessed by scratch wound assay.

RESULTS

Four out of 8 rmAbs strongly reacted with HDF by IFI; Western blotting analysis on HDF membrane extracts showed reactivity with proteins in the range of 35-38 KDa that were further analysed by mass spectrometry.
On the basis of V gene usage and dendrogram analysis, rmAb were subdivided in 3 clusters.
Stimulation of dermal fibroblasts with rmAb induced profibrotic and proinflammatory cytokines production that was dependent on clone clustering; TGF#, IL1#, FGF, IL6 and Rantes are significantly higher in cluster 1 vs 3 ($p=0.02$; $p=0.0018$; $p<0.0001$; $p=0.04$; $p=0.02$, respectively) and in cluster 2 vs 3 ($p=0.01$; $p=0.009$; $p=0.02$; $p=0.019$; $p=0.04$, respectively), with FGF being higher in cluster 1 vs 2 ($p=0.03$). When tested in scratch wound assay, 4/8 IgG4RD rmAbs promoted fibroblast cell migration as compared to control IgGs.

CONCLUSIONS

These results suggest that IgG4-RD patients may develop anti fibroblast antibody that, depending on the V gene usage, are able to modify fibroblast behaviour, potentially driving the fibro-inflammatory process in IgG4-RD patients.

P114

THE FOXO3A/TXNIP AXIS MEDIATES THE INHIBITORY EFFECTS OF THE ANTIEPILEPTIC DRUG LAMOTRIGINE IN TAMOXIFEN-SENSITIVE AND RESISTANT BREAST CANCER CELLS.

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BACKGROUND-AIM

Endocrine resistance constitutes a major challenge in the treatment of estrogen receptor # positive (ER+) breast cancer (BC). Consequently, there is an increasing need to identify novel therapeutic strategies capable of overcoming resistance to hormonal therapies. In this context, the antiepileptic drug Lamotrigine (LTG), which has been shown to induce the expression of the tumor suppressor FoxO3a in Tamoxifen-sensitive and resistant ER+ BC, might be considered as an adjuvant to standard endocrine therapy. Therefore, the aim of this study was to investigate the effects of LTG on the expression of another key tumor suppressor, Thioredoxin-Interacting Protein (TXNIP), which appears to be transcriptionally regulated by FoxO3a.

METHODS

ER+ MCF-7 cells and their Tamoxifen-resistant counterpart (TamR) were employed as experimental models. Additionally, TamR/TetOn-AAA cells — a doxycycline (Dox)-inducible system that expresses a constitutively active form of FoxO3a — and the corresponding control TamR/TetOn-V cells were also employed. Furthermore, these findings were corroborated in two additional ER+ BC cell lines, T-47D and ZR-75, and their Tamoxifen-resistant derivates.

RESULTS

LTG was able to induce ROS production, which in turn has been shown to result in the upregulation of both mRNA and protein levels of FoxO3a and TXNIP in MCF-7 and TamR cells, as well as in T-47D and ZR-75 and their Tamoxifen-resistant counterparts. ChIP assays revealed that LTG promotes the recruitment of FoxO3a to a specific FHRE within the TXNIP promoter in MCF-7 and TamR cells. Furthermore, TXNIP silencing, at least in part, counteracted the inhibitory effect of LTG on cell proliferation in both Tamoxifen-sensitive and resistant BC cells. Additionally, silencing either gene significantly counteracted the inhibitory effects of Lamotrigine on proliferation and migration, and this effect was even more pronounced when both were silenced, particularly in Tamoxifen-resistant cells.

CONCLUSIONS

These data demonstrated that Lamotrigine, for its ability to activate the promising therapeutic targets FoxO3a and TXNIP, might represent a promising candidate for drug repurposing in the treatment of BC, particularly for patients who do not respond to conventional hormonal therapies.

P115

DIFFERENT BCR::ABL1 TRANSCRIPT LEVELS @ 3 AND 6 MONTHS MIGHT BE USEFUL IN IDENTIFYING WARNING/ RESISTANT CML PATIENTS WHEN USING 2G-TKIS AS FRONTLINE TREATMENT

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BACKGROUND-AIM

Second- and third-generation tyrosine kinase inhibitors (2G- and 3G-TKIs) have demonstrated superior efficacy in inducing rapid molecular responses compared to imatinib when used as first-line therapy in patients with chronic myeloid leukemia (CML) in chronic phase (CP). According to current European LeukemiaNet (ELN) recommendations, achieving BCR::ABL1/ABL1^{IS} transcript levels <10% after 3 months or <1% at 6 months of TKI therapy is associated with improved clinical outcomes. These cut-off levels are currently applied uniformly to both 1G- and 2G-TKIs.
In this study we aimed to evaluate the prognostic value of alternative BCR::ABL1 transcript thresholds in predicting treatment outcomes of CP-CML patients receiving 2G-TKIs as first-line therapy and to identify patients candidates to Treatment-Free Remission (TFR).

METHODS

We retrospectively analyzed clinical and molecular responses of 104 CP-CML patients who received 2G-TKIs as frontline therapy at standard dose. The BCR::ABL1/ABL1 levels were measured in blood samples collected at diagnosis and then at three-month intervals using real-time PCR. All molecular analysis were performed in a single reference laboratory.

RESULTS

Patients were stratified based on their response to 2G-TKI according to ELN criteria. At 3 months, the median BCR::ABL1/ABL1^{IS} transcript levels was 0.46% in patients with optimal response, 3.11% in those with non-optimal response and >10% in individual obtaining failure response. ROC curve analysis identified a 3-month threshold of 1.73%. At 6 months, the median transcript levels were 0.043% for optimal responders 0.48% for warning, and 10% for failure, with a ROC-identified threshold of 0.25%. Using this 0.25% cut-off at 6 months, patients with BCR::ABL1/ ABL1^{IS} levels >0.25% showed significantly lower Event Free-Survival (EFS) compared to individual with levels <0.25% ($p < 0.001$). Furthermore, this threshold identified patients with significantly higher likelihood of achieving TFR eligibility.

CONCLUSIONS

Our findings suggest that in CP-CML patients treated with 2G-TKIs as first-line therapy, the use of more stringent molecular thresholds at early time points, particularly at 6 months, can improve prognostic stratification. Specifically, a BCR::ABL1/ABL1^{IS} level of 0.25% at 6 months may represent a useful cut-off to identify patients with a higher probability of achieving EFS and TFR, supporting a more personalized approach to disease monitoring and therapeutic decision-making.

P116

TARGETING OXIDATIVE STRESS IN AML: NEW INSIGHTS FROM SELECTIVE NOX2 INHIBITION

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BACKGROUND-AIM

NADPH oxidases (NOXs) are key sources of reactive oxygen species (ROS) in hematopoietic cells. NOX2 is often overactivated in acute myeloid leukemia (AML), where it supports cell growth, survival, and redox adaptation. Targeting NOX enzymes is emerging as a strategy to disrupt pro-tumoral redox signaling. We examined the effects of MC4876, a selective NOX2 inhibitor, in U937 AML cells with high ROS, to evaluate its impact on cell phenotype and its potential as a redox-targeted therapy.

METHODS

U937 cells were treated with MC4876 (NOX2 inhibitor), MC4921 (NOX2 inhibitor), and MC4953 (NOX1 inhibitor). IC50 values were obtained from viability and cytotoxicity assays at 24, 48, and 72 h. Cell cycle and PI distribution were analyzed by FACS. qPCR assessed NOX1/2 gene expression. Western blot evaluated NOX1/2, cleaved caspase-3/-9, and γH2AX protein levels.

RESULTS

MC4876 showed consistent IC50 values and, at 48 h, activated cleaved caspase-9/-3 and increased γH2AX, indicating intrinsic apoptosis and DNA damage. FACS revealed a modest rise in the pre-G1 population, consistent with early apoptosis or sub-lethal stress. NOX1/2 expression remained unchanged, revealing effects independent of gene modulation, infact we aim to explore downstream redox-sensitive pathways like MAPK/ERK, PI3K/AKT, and NF-κB. MC4921 and MC4953 showed no significant effects.

CONCLUSIONS

MC4876 triggers DNA damage and apoptosis in U937 cells without NOX1/2 downregulation. This points to a non-canonical redox mechanism, possibly involving mitochondrial or cytosolic ROS. Future work will assess other redox targets (e.g., monoamine oxidases) and cell death pathways like ferroptosis. These results support redox-targeted strategies in AML with high oxidative stress.

P117

PATIENT-DERIVED ORGANOIDS TO MODEL DRUG RESPONSE IN PRIMARY LIVER CANCER

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BACKGROUND-AIM

Primary liver cancer (PLC), including hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA), are characterized by a very poor outcome 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 also provide high-throughput experimental techniques to assess the treatments efficacy.
The aim of this study was to treat patient-derived organoids with different agents, screening a library of substances that experimentally affect tumor cells viability, in order to identify candidate therapeutic compounds against PLC.

METHODS

To develop organoids, we minced and digested tumor and non-tumor biopsies into small cell clusters that are seeded into Matrigel. After characterization using immunofluorescence and qPCR techniques, liver organoids were treated with different dosages of each selected compound for 72h, before measuring cell viability. The biological processes potentially targeted by these substances were also studied.

RESULTS

We developed and characterized PLC-derived and healthy organoids, evaluating the morphological characteristics and confirming the presence of typical markers. Subsequently, as an initial screening, we tested their sensitivity to different compounds. In particular, we demonstrated the efficacy of sorafenib (HCC) and gemcitabine+cisplatin (iCCA), proving that organoids represent a reliable model for substances testing. We then analyzed the effect of the natural-derived metabolite Usnic Acid, which may act via MAPK phosphorylation, showing a reduction of cell viability at high concentration. In the context of disease modelling, to better mimic the tumor microenvironment, we co-cultured iCCA-organoids with PBMCs, evaluating the antibody-dependent cell cytotoxicity. In addition, we generated and characterized functional liver organoids, inducing cell differentiation and treating them with oleic acid, in order to develop a model of steatosis.

CONCLUSIONS

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

P118

TARGETING MGLUR5 AND AMPK TO REDUCE HEPATIC STEATOSIS: EVIDENCE FROM HEPG2 CELL AND ORGANOID MODELS

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BACKGROUND-AIM

Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by fatty acid accumulation and can progress to metabolic-associated steatohepatitis and cirrhosis. The metabotropic glutamate receptor 5 (mGluR5) allosteric antagonist, 2-methyl-6-(phenylethynyl) pyridine (MPEP), reduce lipid accumulation and ATP levels in hepatocytes, modulating protein kinase C (PKC) and AMP-activated protein kinase (AMPK) pathways. The aim of this study is to investigate the molecular mechanism underlying the effect of MPEP in steatosis using pharmacological modulators in HepG2 cells and human liver organoids.

METHODS

Steatosis was induced with 2 mM oleate/palmitate for 24 hours. HepG2 cells were treated with mGluR5 negative modulators: MPEP, Fenobam, and CPG (2-Chloro-5-hydroxyphenylglycine), alone or combined with the AMPK inhibitor (Compound C) or a PKC activator (PMA), to assess mGluR5–PKC–AMPK interplay. Non-steatotic cells received the mGluR5 agonist DHPG (Dihydroxyphenylglycine), alone or with MPEP. Lipid and ATP levels, cell viability, and p-AMPK(Thr172)/AMPK ratio were evaluated. Liver organoids were generated from enzymatically digested liver tissue, embedded in Matrigel, and cultured in growth factor-enriched medium. After oleic acid stimulation, organoids were treated with MPEP and lipid content was assessed.

RESULTS

In steatotic cells, MPEP, Fenobam, and CPG reduced lipid accumulation in a dose-dependent manner. In non-steatotic cells, DHPG increased lipid accumulation, an effect reversed by co-treatment with MPEP, confirming mGluR5 involvement, also observed in hepatic organoids. Among the modulators, only MPEP caused ATP depletion and increased the p-AMPK/AMPK ratio. Co-treatment with Compound C attenuated MPEP's effects, suggesting AMPK involvement. Additionally, the lipid-lowering effect of MPEP was abolished by PMA, indicating a role for PKC.

CONCLUSIONS

The use of liver organoids and HepG2 cells demonstrated that selective modulation of mGluR5 receptor affected lipid metabolism and the accumulation of fatty acids in steatosis conditions, highlighting the relevance of this model for studying potential therapies. Furthermore, MPEP modulated lipid metabolism through AMPK activation, induced by ATP depletion, and PKC inhibition.

P119

THE HISTONE DEMETHYLASE KDM3B REPRESENTS A NOVEL EPIGENETIC VULNERABILITY FOR ESTROGEN RECEPTOR-POSITIVE BREAST CANCER

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BACKGROUND-AIM

Approximately 70% of breast cancers (BCs) are hormone-responsive and express estrogen receptor alpha (ERα), which promotes cancer progression by gene transcription through the recruitment of multiprotein coregulator complexes. Histone demethylases (HDMs) modulate the receptor-mediated signaling pathway via histone methylation at ERα target genes. Thus, deciphering how HDMs modify chromatin landscape affecting estrogen signaling may provide new insights into the epigenetic regulation of ER+ BCs. Among these, our attention was caught by the lysine demethylase 3B (KDM3B), that modulates H3K9me2 whose loss has been associated with oncogenic program in ERα+ BCs.

METHODS

We characterized the impact of KDM3B on ERα+ BC models growth and response to antiestrogens by coupling siRNA-mediated gene "knock-down" and pharmacological blockade, using its selective inhibitor, with cellular and functional assays and performing transcriptomic and chromatin accessibility profiling.

RESULTS

We demonstrated the involvement of KDM3B in modulating the transcriptional program of crucial BC pathways, such as cell cycle, cell death and DNA damage response, resulting in a reduced BC cell proliferation and survival upon its depletion or blockade.

CONCLUSIONS

Results obtained demonstrate that KDM3B plays a key role in the ERα epigenetic regulation contributing to the estrogenic signalling cascade, and thus represents a possible novel therapeutic vulnerability for ERα+ BCs.

P120

DRUG DELIVERY OF GLABRESCIONE B BY LIPOSOMES AND ITS THERAPEUTIC EFFECTS ON HEDGEHOG-DEPENDENT TUMORS.

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BACKGROUND-AIM

The Hedgehog (HH) pathway plays a central role in tissue development and its deregulation is implicated in tumorigenesis of several malignancies, including medulloblastoma (MB) and basal cell carcinoma (BCC). MB is a lethal pediatric tumor mainly due to genetic and epigenetic alterations in developmental pathways, including HH signaling. Similarly, mutations in key players of the HH pathway drive BCC, a skin cancer and the most common tumor in the world. Targeting HH signaling represents a promising therapy for HH-driven tumors. In this context, we identified Glabrescione B (GlaB) as the first small molecule able to directly inhibit Gli1, the final effector of HH signaling. GlaB shows strong anti-tumor activity inhibiting MB and BCC growth both in vitro and in vivo, making it a good candidate for preclinical studies. However, GlaB suffers of poor water solubility that limits its bioavailability. This study aims to enhance GlaB solubility, efficacy and delivery by using liposomal formulations.

METHODS

Luciferase reporter assays, Western blotting, and RT-qPCR were conducted to investigate the effect of GlaB encapsulated in liposomes (Lipo/GlaB) on Gli1 transcriptional activity and expression. Proliferation assays were performed on primary murine SHH-MB and BCC cells to assess the impact of Lipo/GlaB on tumor growth in vitro. The therapeutic efficacy of Lipo/GlaB was evaluated in in vivo models of MB and BCC. Tumor masses were analyzed by Western blotting, RT-qPCR, and immunohistochemistry.

RESULTS

We demonstrated the high ability of Lipo/GlaB to inhibit the Gli1 transcriptional activity at a lower concentration (IC50 1.08μM) compared to GlaB (IC50 12μM). Lipo/GlaB strongly decreases the proliferation of HH-driven MB and BCC cells, as the consequence of the reduction of HH signature. Notably, these data have been confirmed in in vivo models of both MB and BCC in which the treatment with Lipo/GlaB robustly reduces the tumor growth, by inhibiting Gli1 expression.

CONCLUSIONS

These findings clearly support the antitumor properties of Lipo/GlaB for the treatment of HH-driven malignancies, such as BCC and MB. Liposomal encapsulation of GlaB improves its therapeutic efficacy, thus underlining the relevance to develop engineered nanocarriers for HH inhibitors to ameliorate their therapeutic outcome.

P121

ACTIVATION OF AURORA A/B PATHWAY IN PRECLINICAL MODELS OF NSCLC CELLS CARRYING PI3KCA MUTATIONS

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BACKGROUND-AIM

Mutations of the p110α subunit of class I phosphatidylinositol 3-kinase (PIK3CA) gene have been detected in 4-6% of lung adenocarcinoma patients (LUAD).
These mutations, which typically affect the helical binding domain (exon 9, E545K, or E542K) or the catalytic subunit (exon 20, H1047R, or H1047L), are considered oncogenic and targetable. Despite their well-established oncogenic role, no therapies are currently approved. The aim of the present project is to identify and understand the role of these mutations in activating alternative signalling pathways, and to explore targeted therapeutic options.

METHODS

H322 and H292 cells were transfected with plasmids carrying PIK3CA H1047R or E545K mutations. The cell growth and migration were evaluated by cell counting and transwell migration assay. The intracellular signaling was tested by western blot analysis. The most effective drug that can synergize with PI3K inhibitors was assessed with a panel of 176 drugs by a mid-throughput drug screening approach. The combination of the drugs was evaluated by Combenefit software analysis. The apoptotic cells were detected using the Annexin V assay. The colony assay was evaluated by crystal violet.

RESULTS

PIK3CA-mutated cells showed increased growth rate and enhanced migration associated with an increased activity of the PI3K/Akt/mTOR pathway and acquisition of a mesenchymal phenotype. We used a mid-throughput analysis drug screening approach to identify the best biologically active molecules as candidates for combination with drugs directed towards specific PI3K mutations. This analysis revealed that the Aurora kinase inhibitor was the most promising compound for combination with the PI3K inhibitor. The combined inhibition of the PI3K and AURORA A/B pathways reduced cell proliferation synergistically in clones harbouring H1047R and E545K mutations in both the H322 and H292 cell lines. This combination impaired colony formation and cell migration, promoting apoptosis via the inhibition of PI3K/Akt and Aurora kinase B.

CONCLUSIONS

In this study, we reported that combining PI3K and Aurora Kinase A/B inhibitors could be an effective way of treating NSCLC cells with PIK3CA mutations and could improve treatment outcomes.

P122

"ANTI-INFLAMMATORY EFFECTS OF INHALABLE ILOPROST FORMULATIONS IN PRIMARY HUMAN CYSTIC FIBROSIS NASAL EPITHELIAL CELLS "

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BACKGROUND-AIM

To develop effective interventions for the hyperinflammatory state associated with Cystic Fibrosis (CF), we established a robust human cell culture model that closely mimics the in vivo airway epithelium. We used this platform to assess the cytocompatibility and anti-inflammatory efficacy of smart drug-delivery systems, specifically inhalable mucopenetrating Nano-in-Micro (NiM) formulations carrying a repurposed anti-inflammatory agent (doi: 10.1021/acsanm.4c01379).

METHODS

To date, nasal epithelial brushings have been collected from 10 individuals with CF. The isolated cells were expanded using the conditional reprogramming culture method and subsequently differentiated into air-liquid interface (ALI) cultures capable of producing endogenous mucus. The transepithelial electrical resistance (TEER) was measured by a voltmeter. These ALI cultures were exposed to Pseudomonas aeruginosa lipopolysaccharide (LPS), either alone or in combination with NiM formulations—pegylated or non-pegylated—carrying Iloprost (ILO), a prostacyclin analogue. The expression levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-8), as well as inflammation-associated microRNAs (miR-145, miR-146a, miR-17), were quantified by real-time PCR. Fluorescein Isothiocyanate (FITC)-conjugated NiM formulations were employed for cellular uptake studies.

RESULTS

The increase in TEER, measured during ALI conditions, testifies the generation of a tight epithelium. The treatment with NiM-PEG-ILO significantly attenuated the expression of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β compared to LPS stimulation alone. Furthermore, a marked downregulation of miR-145, miR-146a, and miR-17 levels was observed relative to the LPS-only group. NiM-PEG-ILO were also more efficient than NiM-ILO. Cytofluorimetry analysis showed that both NiM formulations, NiM_FITC and NiM_PEG_FITC were internalized by cells in a concentration-dependent manner.

CONCLUSIONS

Our results demonstrate that Iloprost, already used to treat pulmonary arterial hypertension, can be vehicled efficiently to CF airway epithelia in a pre-clinical model of airway epithelium in order to attenuate inflammation. Moreover, higher efficiency of NiM-PEG-ILO than non-pegylated formulation may be due to mucopenetrating properties rather than to increased cell uptake.

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P123

AXL INHIBITOR TP-0903 HAS AN IMMUNOMODULATORY EFFECT ON MESOTHELIOMA CELLS THUS RENDERING THEM MORE SUSCEPTIBLE TO NATURAL KILLER CELLS

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BACKGROUND-AIM

The identification of molecular mechanisms making tumor cells more sensitive to immune surveillance is a significant challenge for onco-immunologists to optimize current immunotherapies. In this context, malignant mesothelioma (MMes) is still a hard-to-treat cancer that requires emerging treatment options. Thus, we explored a novel immunomodulatory mechanism to induce and promote a more effective recognition and killing of MMes cells by Natural Killer (NK) cells. Accordingly, we explored the immunomodulatory effect of TP-0903 or dubermatinib, on the expression of ligands for NK cell-activating receptors in MMes cells and thus on their recognition and killing mediated by NK cells. TP-0903 is an AXL inhibitor that blocks the formation of the MDM2–MDMX complex, induces MDM2 destabilization, increases p53 function and thus should increase ligands for NK cell-activating receptors.

METHODS

In vitro TP-0903 treatment of MMes cell lines was performed: i) to evaluate a pre-apoptotic dose of TP-0903 allowing MMes cells to be recognized by NK cells, ii) to assess the effect of TP-0903 on p53 function by evaluating p53 target genes, including MDM2 and p21, in MMes cells by western blotting analysis, iii) to evaluate the expression of ligands for NK cell activating receptors, apoptotic death receptors, and ICAM-1 by flow cytometry, iv) to investigate the NK cell-mediated anti-tumor response through functional assays of apoptosis and degranulation against TP-0903-treated MMes cells.

RESULTS

Treatment of MMes cells with TP-0903 used at pre-apoptotic dose resulted in increased expression of i) ligands for NKG2D and DNAM-1 activating receptors and ICAM-1 adhesion molecule, which mediated enhanced NK cell degranulation, ii) pro-apoptotic molecules, which potently boosted NK cell-mediated apoptosis of MMes cells.

CONCLUSIONS

These results demonstrated the immunomodulatory effect of TP-0903 which makes MMes cells more susceptible to recognition and killing by NK cells, thus providing a novel tool to boost NK cell-mediated killing of MMes cells. These findings might lead to the development of new NK cell-based immunotherapy strategies for the treatment of malignant mesothelioma.

P124

DECODING A FOUNDER MUTATION: PRECISION SCREENING IN AN ISOLATED ITALIAN POPULATION

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BACKGROUND-AIM

Pathogenic variants in the LMNA gene are associated with cardiomyopathy and neuromuscular disease, often leading to life-threatening arrhythmias and heart failure. Genetic isolates provide unique opportunities to study private mutations and their clinical impact. We investigated the prevalence, clinical phenotype, and care implications of a novel LMNA founder mutation identified in a geographically isolated Italian village, applying a multidisciplinary and community-engaged screening model.

METHODS

We conducted an observational, prospective study involving clinical researchers, IT specialists, and local citizens. Genealogical reconstruction identified 2,948 individuals over 12 generations. From 47 family branches, 251 living and contactable relatives were identified; 234 (93%) underwent full genetic and clinical assessment. Systematic screening included ECG, echocardiography, and cardiac magnetic resonance (CMR). AI-based models were developed to predict LMNA carrier status. A historical cohort of 25 sporadic LMNA cases was used for comparison.

RESULTS

A novel truncating LMNA mutation (c.208del; p.Val70Serfs*26) was identified in 30 carriers (12.8%), clustered in two branches. All carriers exhibited cardiac abnormalities, and 43% had neuromuscular signs. Despite mild symptoms in 47%, CMR detected early disease in 100%. At a median 31-month follow-up, 13% experienced major adverse events (MAEs: cardiac death, transplant, malignant arrhythmias). Compared to sporadic cases, founder carriers had higher LMNA-VTA scores and more frequent MAEs. AI predicted genotype with 90% accuracy using biometric and ECG data.

CONCLUSIONS

This study demonstrates that founder mutation screening in genetic isolates, supported by citizen engagement and multidisciplinary care, can lead to early diagnosis, improved risk prediction, and life-saving interventions in rare cardiac disease.

P125

FGF/FGFR SYSTEM IN MEDULLOBLASTOMA: NEW APPROACHES FOR TUMOR TREATMENT.

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BACKGROUND-AIM

Medulloblastoma (MB) is a highly aggressive childhood brain tumor, accounting for about 20% of pediatric brain cancers. It mostly affects children aged 3–9 years but can also occur in adults. Symptoms often include cerebellar dysfunction, increased intracranial pressure, and hydrocephalus. MB is divided into four groups based on genomic features: WNT (MB-WNT), SHH (MB-SHH), group 3 (MB-Gr3), and group 4 (MB-Gr4), which differ in genetics, outcomes, and risk. Each group can be further divided into subtypes, which impact prognosis and therapy. Current treatments involve surgery, radiotherapy, and chemotherapy, but recurrence and mortality rates remain high, with 5-year survival ranging from 55% to 95% depending on the subtype. MB-Gr3 accounts for ~30% of cases, mostly affects infants and young children, and has the worst prognosis (5-year survival <30%), especially with MYC amplification. Studies show that the FGF/FGFR pathway drives cancer growth, invasion, angiogenesis, and therapy resistance. Although FGFR inhibitors (FGFRi) are approved for other cancers, their role in MB is unexplored. This study aims to evaluate FGFRi and novel FGF traps developed in our lab as a therapeutic strategy for aggressive MB-Gr3.

METHODS

In this study we exploited MB-Gr3 cells (HD-MB03, D425Med, MED411 and MED2112) to assess the expression and activation of the FGF/FGFR system. The inhibition of FGF/FGFR was carried out using a clinical grade FGFR inhibitor (Erdafitinib) and two FGF trap small molecules (NSC12 and UPR1430). Cell proliferation, Western blot and flow cytometry were used to test the effect of the drugs. In vivo, orthotopic tumor xenograft was performed to validate the therapeutic effect and immunohistochemistry for the analysis of tumor samples.

RESULTS

Preliminary data confirm that FGFRs are highly expressed and activated in aggressive MB subtypes, correlating with worse prognosis. In vitro studies demonstrate that both FGFRi (Erdafitinib) and the FGF traps (NSC12 and UPR1430) significantly reduce MB cell proliferation. Indeed, Western blot analyses confirmed that anti-FGF/FGFR treatments significantly reduced the activation of FGFR and levels of MYC. In vivo, FGFR inhibition resulted in reduced tumor growth and impaired intra-tumor angiogenesis in an orthotopic model of MB-Gr3 (HD-MB03 cells).

CONCLUSIONS

In conclusion, our preliminary data suggest that the FGF/FGFR system plays a role and might represent a promising target for the treatment of MB-Gr3.

P126

NANOPLASTICS AND THE BONE MICROENVIRONMENT: OXIDATIVE STRESS AND EV SIGNALING IN MULTIPLE MYELOMA.

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BACKGROUND-AIM

Plastic pollution has become one of the most recognized global environmental and public health issues. While the presence of micro- and nanoplastics (NPs) in aquatic and terrestrial environments is well documented, limited data exist on the effects of NPs on human health. Due to their small size, NPs penetrate biological tissues and disrupt essential cellular functions. Their toxicity is mainly linked to oxidative stress (OS), a known contributor to bone remodeling impairment. As the bone microenvironment (BME) is a key niche for the development of certain cancers, this study investigates the impact of NPs in both physiological and pathological conditions, with a focus on multiple myeloma (MM), where extracellular vesicles (EVs) play a crucial role in tumor niche education. We also assessed how NP-induced OS affects EV-mediated communication between BME and MM cells.

METHODS

The MC3T3-E1, MLOY-4, RAW 264.7, OPM2, HS5, and MC3T-E1 cell lines were used for the in vitro studies. Cells were exposed to NPs (1-200 μg/ml) for 48 hours. 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 cytometer, and cryo-EM.

RESULTS

Murine bone cells were first exposed to NPs, revealing reduced viability, increased ROS levels, and caspase 3/7 activation, consistent with the induction of apoptosis. NPs also impaired pre-osteoblast migration, stimulated osteoclastogenesis, and altered gene expression of inflammatory and osteogenic markers, indicating a disruption in bone remodeling.

In parallel, human stromal and MM cells treated with NPs for 48 hours exhibited reduced viability and proliferation, increased ROS, and upregulation of antioxidant response genes. Moreover, NPs modified the concentration, size, and morphology of MM-derived EVs. When pre-osteoblasts were treated with these NP- EVs, functional changes were observed, suggesting that NPs affect vesicle content and their ability to support MM progression.

CONCLUSIONS

Overall, NPs appear to disrupt skeletal homeostasis and modulate EV-mediated communication in the BM, providing new insights into their potential role as modulators of this process.

P127

"NEW STRATEGIES TO IMPROVE CANCER IMMUNOTHERAPY THROUGH THE REGULATION OF PD-L1 ENDOCYTOSIS BY UPAR ANTAGONIST PEPTIDES"

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BACKGROUND-AIM

Immunotherapy has revolutionized cancer treatment and revitalized the field of tumor immunology. Recent evidence has demonstrated the clinical efficacy of programmed cell death-1 (PD-1) and programmed death ligand-1 (PD-L1) antibodies in patients with metastatic breast cancer, melanoma, and non-small-cell lung cancer. The therapeutic effectiveness of PD-1/PD-L1 inhibitors is particularly pronounced in patients exhibiting high PD-L1 expression. Recently, Tseng et al. demonstrated that targeting Plasminogen Activator Inhibitor-1 (PAI-1) with its inhibitor tiplaxtinin (TPX) synergizes with anti-PD-L1 checkpoint blockade in a murine melanoma model. PAI-1 induces the internalization of surface PD-L1, leading to a reduction of PD-L1 expression at the cell membrane. The binding of PAI-1 to the uPA/uPAR complex facilitates the recruitment of low-density lipoprotein receptor-related protein 1 (LRP1), which triggers an endocytic process resulting in the internalization of the PAI-1–uPA–uPAR–LRP1 quaternary complex. We propose to inhibit PDL-1 endocytosis by uPAR inhibitors to maintain high-cell-surface levels of PD-L1. Moreover, we propose to set up 3D co-culture system between non-small cell lung cancer cells and T cells to assess efficacy of uPAR inhibitors on immunotherapy responses.

METHODS

2D and 3D cultures from A549 (non-small cell lung cancer) and BXPC3 (pancreatic cancer) were treated with TPX and uPAR antagonist (IPR803) to evaluate the effect of TPX and IPR803 treatment on PD-L1 modulation. Surface PD-L1 expression were evaluated by FACS, confocal microscopy and western blotting. 3D co-cultures were seeded between A549 and BXPC3 previously treated with anti-human PD-L1 and IPR803 and T cells CD8+ CD4+ to asses cytotoxic effect of T cells on tumor cells. Cytotoxicity activity of T cells were evaluated with Live/Dead staining and PrestoBlue assay.

RESULTS

Our result evidenced that in 2D and 3D cultures of A549 and BXPC3 TPX and uPAR antagonist peptides are able to block the PD-L1 internalization and, consequently, to increase PD-L1 membrane levels. In parallel, our data highlighted in 3D co-cultures an improvement of cytotoxic effect of T cells on A549 treated with uPAR antagonists and anti-PD-L1 antibodies.

CONCLUSIONS

Our data demonstrated that uPAR inhibition by uPAR antagonist peptides result in a significant increase in surface PD-L1 levels, opening the way for new combined therapeutic strategies with uPAR inhibitors and anti-PD-1/PD-L1

P128

ICOS-FC INHIBITS SPHEROID GROWTH AND TUMOUR-PROMOTING PATHWAYS IN A 3D BREAST CANCER MODEL

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BACKGROUND-AIM

Inducible Co-Stimulator (ICOS/CD278), a costimulatory molecule expressed on activated T cells, triggers co-stimulatory signals that promote activation, cytokine production, inflammation, cell migration, angiogenesis, and T cell differentiation. Its interaction with the ICOS Ligand (ICOSL) not only regulates the immune response but also transduce intracellular signals in the cells expressing ICOSL, a phenomenon referred to as “reverse signaling”. In tumours, ICOSL activation by ICOS-Fc, a recombinant protein composed of the extracellular portion of ICOS fused with the IgG1 Fc domain, has been shown to inhibit cell migration, metastasis, and angiogenesis. While its therapeutic potential has been explored in several solid tumours, the effect of ICOS-Fc on breast cancer remains largely uncharacterized. This in vitro study aimed to evaluate the biological activity of ICOS-Fc in a 3D breast cancer model using 4T1 murine mammary carcinoma cells, focusing on its effects on tumour spheroid development.

METHODS

Luciferase-expressing 4T1 breast cancer cells were seeded (20,000 cells/well) in 96-well plates pre-coated with Matrigel to promote 3D spheroid formation. Treated groups received ICOS-Fc (2, 4, or 10μg/ml) mixed with Matrigel, while controls received Matrigel with media only. Spheroid growth was monitored for 7 days and imaged daily using phase-contrast microscopy. Moreover, Real Time-PCR analysis was performed on day 3 to evaluate the expression of markers associated with inflammation, angiogenesis, and survival, including IL-1β, IL-6, TNF-α, VEGF, TGF-β, BCL2, and BCL2L1.

RESULTS

By day 7, ICOS-Fc treatment resulted in visibly smaller and more compact spheroids in a dose-dependent manner, particularly in the 4 and 10μg/ml groups. Molecular analysis revealed significant downregulation of pro-inflammatory (IL-1β, IL-6, TNF-α), pro-angiogenic (VEGF, TGF-β), and anti-apoptotic (BCL2, BCL2L1) markers.

CONCLUSIONS

These findings highlight the potential of ICOS-Fc as an effective agent in breast cancer treatment. Its ability to impair spheroid development by downregulating key pro-inflammatory, angiogenic, and survival markers suggests a promising antiproliferative and immunomodulatory role, warranting further investigation in vivo.

P129

PMA SENSITIZATION OF SH-SY5Y NEUROBLASTOMA CELLS TO JURKAT CELL-MEDIATED, TRAIL-INDUCED APOPTOSIS IS ENHANCED BY TRKAIII.

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BACKGROUND-AIM

Neuroblastomas (NBs) are heterogeneous, aggressive, therapy-resistant embryonal tumors that originate from cells of neural crest origin committed to the sympathoadrenal progenitor cell lineage, represent the most common extracranial solid tumor of childhood and account for ≈15% of pediatric cancer-related deaths. Advances in multimodal therapy have increased 5-year survival rates to ≈50% in high-risk NB but have reached a plateau, prompting novel initiatives to harness the immune system. NBs, however, are immunologically "cold" tumors and, in such, represent an immunotherapeutic challenge.
This study aims to characterize mechanisms that sensitize NB cells to immune-mediated apoptosis, focusing on the role of TrkAIII expression and PKC activation.

METHODS

Using live cell imaging, adhesion assays, indirect immunofluorescence, Western blotting, RT-PCR, and a variety of inhibitors, we investigated the effect of the PKC activator phorbol myristate acetate (PMA) on SH-SY5Y NB cells expressing low (pcDNA-SH-SY5Y) and high (TrkAIII-SH-SY5Y) levels of TrkAIII, and their sensitivity to Jurkat cell-mediated TRAIL-induced apoptosis.

RESULTS

Low (pcDNA-SH-SY5Y) and high (TrkAIII-SH-SY5Y) TrkAIII expressing SH-SY5Y NB cells, resistant to Jurkat cell-mediated elimination under normal conditions, become sensitive to Jurkat cell-mediated TRAIL-induced apoptosis following pre-treatment with the PKC activator PMA, and that this sensitivity is markedly enhanced by TrkAIII expression. Sensitization to Jurkat-induced apoptosis involved a switch from SH-SY5Y to Jurkat LFA1-dependent adhesion and the formation of LFA1/ICAM1 lipid-raft immune synapses, with Jurkat membrane (m)TRAIL-induced apoptosis associated with PMA enhanced SH-SY5Y DR5 expression. This mechanism was granzyme B-independent, not regulated by PD-L1/PD-1 despite SH-SY5Y PD-L1 expression, and was augmented by TrkAIII-cFLIP sequester.

CONCLUSIONS

Together, the data characterize a novel molecular mechanism for sensitizing TrkAIII expressing and non-expressing NB cells to immune cell-mediated mTRAIL-induced apoptosis, providing a basis for future evaluation of autologous LFA1 and TRAIL-expressing NK or γ/δ T cells, and clinically approved PKC activators (e.g. Bryostatin 1), in the further development of this novel potential immunotherapeutic strategy for "cold" TrkAIII expressing and non-expressing NBs.

P130

STRESS-INDUCED TRKAIII ACTIVATION AUGMENTS PD-L1 EXPRESSION IN SH-SY5Y NEUROBLASTOMA CELL BY ACTIVATING β-CATENIN

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BACKGROUND-AIM

Neuroblastomas (NBs) are heterogeneous, aggressive, therapy-resistant embryonal tumors, originate from cells of neural crest origin committed to the sympathoadrenal progenitor cell lineage, represent the most common extracranial solid tumor of childhood and account for ≈15% of pediatric cancer-related deaths. Metastatic NB progression correlates with expression of the oncogenic alternative neurotrophin receptor tropomyosin-related kinase A (TrkA) splice variant, TrkAIII, elevated expression of the immune checkpoint protein PD-L1 and the activation of ß-catenin signaling.

METHODS

In this study, we evaluated the possibility that TrkAIII may regulate PD-L1 expression and ß-catenin signaling in an SH-SY5Y NB model, by RT-PCR, Western blot, and indirect IF, and by Jurkat IL-2 production assay.

RESULTS

Trk (entrectinib), PIP3K (LY294002), Akt (capivasertib) and ß-catenin (FH355) inhibitors revealed that under normal conditions, cell cycle-regulated ligand-independent TrkAIII activation enhanced constitutive functional PD-L1 expression through PI3K/Akt but not activate ß-catenin. In contrast, ER stress induced by DTT, which promotes TrkAIII mitochondrial importation and activation, markedly augmented PD-L1 expression via PIP3K/Akt and ß-catenin signaling pathways, in association with nuclear accumulation of a 63kda S552-phosphorylated ß-catenin CT-fragment.

CONCLUSIONS

These observations link ER stress-induced TrkAIII activation to the activation of ß-catenin and promotion of PD-L1 expression, implicate ß-catenin in stress-regulated TrkAIII oncogenic activity, add PD-L1-mediated immune evasion to TrkAIII's oncogenic repertoire and help to explain co-association between alternative TrkAIII splicing, PD-L1 expression, ß-catenin activation and metastatic NB progression.

P131

INSIDE THE MECHANISM: NEW INSIGHTS ABOUT THE ROLE OF GLUTATHIONE TRANSFERASE OMEGA 1-1 (GSTO1-1) IN CANCER RESISTANCE AGAINST ARSENIC TRIOXIDE (ATO).

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BACKGROUND-AIM

The role of GSTO1-1 and its polymorphisms in the metabolism of ATO – both as an environmental contaminant and a chemotherapic drug - has been suggested by numerous studies [1]. We have previously demonstrated that GSTO1-1 can play a pivotal role in ATO metabolism by cancer cells, increasing both sensitivity and resistance, depending on its expression levels [2]. Given the modulatory action of GSTO1-1 on pro-suvival pathways involved in cisplatin resistance [3], the aim of this study was to provide an in-depth analysis of the molecular mechanisms connecting GSTO1-1 expression with cancer cells resistance against ATO.

METHODS

HeLa GSTO1-1 stable transfected (HeLaGSTO1+), HeLa control (HeLaCont) and HeLa CRISPR/Cas 9 ko (HeLaGSTO1-) cells were used as a model of cervical carcinoma expressing different levels of GSTO1-1. Cell viability was assessed by Alamar blue assay; autophagy was evaluated by acridine orange staining and LC3 immunofluorescence (IF). ROS production (DCFDA), subcellular alterations (TEM equipped with immunogold labeling) and genomic instability (H2AX phosphorylation) were also evaluated. MAPKs pathways involved in cell survival and proliferation were analyzed by immunoblot.

RESULTS

Cells overexpressing GSTO1-1 showed a significant increase in resistance against ATO treatment, along with elevated levels of AKT and ERK1/2 phosphorylation, while JNK phosphorylation was inhibited. No correlation was observed between GSTO1-1 overexpression and ROS production or autophagy induction. TEM revealed the presence of numerous vacuoles in cells upon ATO treatment and HeLaGSTO1+ cells showed a significant increase in H2AX phosphorylation.

CONCLUSIONS

Our results confirm that high levels of GSTO1-1 expression confer resistance to ATO. This effect, rather than a consequence of the direct action of GSTO1-1 on ATO, is due to the modulation of intracellular pro-survival pathways, namely AKT and ERK1/2, and may be associated with genomic instability as well. These data futher confirm a more general role of GSTO1-1 in defense mechanisms of cancer cells, going beyond the classical detoxification role played by glutathione transferases.

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[1] Belcastro E. et al., under review
[2] Piaggi S. et al., under review
[3] Piaggi S. et al. Carcinogenesis. 2010; 31: 804

P132

OBESITY-ASSOCIATED EXTRACELLULAR VESICLES ENHANCE FIBROBLAST TUMOR-PROMOTING FUNCTIONS IN BREAST CANCER VIA LET-7A/FGF19 AXIS

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BACKGROUND-AIM

Obesity significantly impacts breast cancer (BC) incidence, prognosis, and therapy response. It induces both systemic and local alterations that reshape the BC microenvironment, in which the dominant cell types are fibroblasts. The mechanisms behind this interplay are multifactorial and involve different mediators, among which extracellular vesicles (EVs), small membrane particles secreted by various cells, are emerging as key actors. However, the specific role of obesity-derived EVs in affecting tumor-stimulatory properties of stromal fibroblasts remains poorly understood.

METHODS

EVs were isolated from the serum of healthy normal-weight (NW: body mass index (BMI) <24.9kg/m^2) and overweight/obese (OW/Ob: BMI>25kg/m^2) women and characterized according to MISEV23 guidelines. Immortalized mammary control fibroblasts (CFs), experimental cancer-associated fibroblasts (exp-CAFs), and primary human CAFs (hCAFs) derived from BC biopsies were employed as stromal models, while MCF-7 and T47D cells were used as BC models in different biological assays.

RESULTS

Treatment with OW/Ob-EVs promoted pro-tumor phenotype of CFs, increased the tumor-promoting activities of CAFs and positively modulated the tumour/stroma crosstalk in co-culture experiments. Cytokine array revealed that treatment of CFs with OW/Ob-EVs was associated with an enhanced release of Fibroblast Growth Factor 19 (FGF19), confirmed by ELISA and qRTPCR. Pharmacological/genetic inhibition of FGF19 in fibroblasts reversed the described pro-tumorigenic effects of OW/Ob-EVs in terms of motility, contractility and crosstalk with BC cells. Our previous studies revealed a BMI-dependent EV-miRNA signature, with let-7a significantly down-regulated within EVs of OW/Ob patients. Of note, 'in silico' prediction identified FGF19 as a let-7a target, and let-7a was also found reduced in the current OW/Ob-EV population. The impact of let-7a in mediating OW/Ob-EV effects on FGF19 expression and on fibroblast functions was further investigated using let-7a mimic and inhibitor.

CONCLUSIONS

Our findings offer new insights into how obesity-derived EVs affect tumor-stroma interactions and BC progression, highlighting the let-7a/FGF19 axis as a potential therapeutic target.

P133

UNVEILING THE LINK BETWEEN THE MEDITERRANEAN DIET AND BREAST CANCER: THE EMERGING ROLE OF EXTRACELLULAR VESICLES

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BACKGROUND-AIM

Environmental factors, particularly those related to diet and specific nutrients, play a significant role in breast cancer (BC) biology. There is a growing interest in the potential benefits of diet and exercise in preventing and treating BC. Among dietary approaches, the Mediterranean Diet (MD) is most effective in lowering BC risk and improving outcomes. Numerous studies have attempted to address the relationship between diet and circulating Extracellular Vesicles (EVs), key mediators of intercellular communication in BC. It has been reported that dietary compounds may influence the biogenesis, release, and composition of the endogenous EVs. However, there is still a knowledge gap regarding the accurate phenotyping of diet-induced EVs and their potential role in mediating the effects of nutritional status on BC. We investigated the impact of MD adherence on the composition and function of serum circulating EVs isolated from BC patients.

METHODS

EVs were isolated and characterized, according to the MISEV 2023 guidelines, from the serum of BC patients, grouped by their adherence to the MD (Low: ≤5; Medium: 6–9; High: ≥10), as assessed by the MEDAS questionnaire. The effects of isolated EVs on cell proliferation, stemness and motility were evaluated in two different ERα-positive BC cell lines, MCF-7/T-47D. The expression profiles of EV-packaged miRNAs by SmallRNA-sequencing analysis were assessed.

RESULTS

We found that High MD adherence was associated with changes in the number and size of circulating EVs in BC patients. EVs from patients with High MD adherence were less effective in promoting growth, migration and stemness of ER+ BC cells than EVs from patients with Low MD adherence. The EV-miRNA analysis identified a total of 172 miRNAs, 18 of which were differentially expressed (#fold change#≥1.5) in Low vs High MD adherence EVs. These are mainly involved in regulating proliferation, migration and metabolism in BC.

CONCLUSIONS

These findings support the hypothesis that High MD adherence modulates the EV composition (e.g. the miRNA profile) and exerts beneficial effects on BC behaviour. Although these results are preliminary, they underscore the importance of healthy dietary patterns in modulating BC biology and open new avenues for identifying potential therapeutic targets, mainly in patients with poor dietary habits.

P134

THE ANTITUMORAL EFFECT OF PIRFENIDONE ON MELANOMA CELLS

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BACKGROUND-AIM

Melanoma represents one of the most aggressive cancers with incidence and mortality rates continuously increasing. Despite the initial responses to therapies available to date, disease progression remains difficult to manage. Thus, there is an urgent need for novel and unexplored therapeutic strategies. Pirfenidone (PFD) is an antifibrotic drug that has recently been proposed for the treatment of solid cancers. Interestingly, besides its direct antifibrotic effect on mesenchymal-derived cells of the tumor microenvironment, PFD has shown antitumoral properties. We investigated the antitumoral effect of PFD in a preclinical model of melanoma highlighting for the first time the different signalling network underlying this antineoplastic effect.

METHODS

Two different human melanoma cell lines (A375M6 and M21) were exposed to PFD at two different concentrations (2.7 mM and 8.1 mM), reflecting the doses used in patient treatment. The effect of PFD on melanoma cell has been evaluated using MTT assay, colony forming assay, invasiveness through Geltrex™ pre-coated filters, and western blotting assay for TGFβ1 and pSMAD2 expression. Moreover, we explore the anti-oxidant properties of PFD using ROS quantification, and finally we correlated the mechanism of PFD activity on furin enzymes.

RESULTS

We found that PFD alone inhibits melanoma cell proliferation, and this is associated with the increase in p21 expression in a dose dependent manner, also the clonogenic activity was significantly reduced. PFD reduces intracellular ROS and inhibits TGFβ1 signalling, promoting its accumulation as intracellular inactive propeptide, probably due to a direct inhibition of furin enzyme that is involved in TGFβ1 processing. Indeed, the use of furin inhibitor, revealed a significant reduction on melanoma cells proliferation and the co-treatment with furin inhibitor and PFD revealed a strong synergism on melanoma cell proliferation and induction of apoptosis.

CONCLUSIONS

We investigated a previously unexplored mechanism of PFD activity on cancer cells, and we strengthened the notion that PFD may improve advanced metastatic melanoma treatment in a repurposing modality.

P135

UNVEILING MOLECULAR AGING MECHANISMS: RELATIVE TELOMERE LENGTH AND INFLAMMATION-ASSOCIATED PATTERNS

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BACKGROUND-AIM

Telomere attrition and chronic inflammation are hallmarks of aging [1], and their progression may impact the quality of the aging process [2]. This study examines the relationship between relative leukocyte telomere length (LTL), key inflammatory markers, and chronological age, as molecular signatures associated with longevity [3].

METHODS

A total of 63 healthy individuals were selected from previous studies based on age, sex, and the availability of blood and serum samples. Participants were categorised into five age groups: adults (40–64 years, n = 15), older adults (65–89 years, n = 15), nonagenarians (90–99 years, n = 10), and centenarians, including semi- and supercentenarians (100 years and older, n = 23). Relative LTL was assessed from blood-derived DNA using qPCR, based on a modified method by Cawthon (2002) [4,5], and calculated as the exponential ratio between telomere repeats and a single-copy gene (36B4), using a reference DNA as calibrator. Serum levels of TNF-α, IL-10, and IL-1β were quantified via ELISA.

RESULTS

The data showed a non-significant negative correlation between LTL and age, with a more evident inverse trend in females, likely due to their higher representation. No significant differences in LTL were observed across age or sex groups, except in nonagenarians, who showed higher LTL, a pattern confirmed only in females. Cytokine analysis revealed a significant age-related increase in TNF-α, peaking in centenarians, and a rising trend in IL-10, reflecting shifts in inflammatory balance. IL-10 levels were significantly higher in nonagenarians compared to adults. IL-1β was undetectable in all samples except one.

CONCLUSIONS

The age-related decline in LTL reflects biological aging, while higher LTL in nonagenarians may indicate successful aging [6]. The female-specific trend supports the greater longevity in women, suggesting potential gender-related protective factors. Increased TNF-α with age supports the concept of inflammaging [7], while elevated IL-10 may represent a compensatory anti-inflammatory response. These findings deepen understanding of telomere and immune changes with aging and highlight the role of sex-specific factors and extreme longevity in aging biomarker research.

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P136

INVESTIGATING IMPORTIN α ISOFORMS INVOLVED IN ERK5 NUCLEAR TRANSLOCATION AS POTENTIAL THERAPEUTIC TARGETS IN MELANOMA

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BACKGROUND-AIM

ERK5 (Extracellular-signal Regulated Kinase 5) is emerging as a promising target in cancer therapy. ERK5 pro-proliferative activities are linked to its nuclear localization. Cargo proteins with high molecular weight and classical nuclear localization signal (cNLS) require active transport to enter into the nucleus. This is mediated by heterodimeric importins. Importin α subunit binds the cNLS and importin β1 interacts with nucleoporins to mediate nuclear entry. ERK5 has both a large size and a C-terminal cNLS. We recently identified importin β1 as a key mediator of ERK5 nuclear shuttling and showed that its inhibition synergizes with ERK5 inhibitors to markedly reduce cancer cell proliferation. This study was addressed to identify which importin α isoform(s) (encoded by human KPNA1-7 genes) is involved in ERK5 nuclear import.

METHODS

TCGA melanoma datasets were analyzed using TNMplot and cBioPortal to assess expression of importin α isoforms in tumor vs normal tissue and their correlation with ERK5 targets. We performed siRNA-mediated knockdown (KD) of importin α isoforms, and evaluated the amount of nuclear ERK5 by confocal immunofluorescence in unstimulated (routinely cultured melanoma) and stimulated (EGF-treated HeLa, a widely used model for cyto-to-nuclear translocation studies) cells. The effects of importin α silencing on cell viability were evaluated by measuring protein content in cell lysates.

RESULTS

In silico analysis revealed that KPNA2 mRNA is significantly upregulated in several cancers, including cutaneous melanoma, with higher levels in both primary and metastatic tumors compared to normal tissue. KPNA2 expression correlated with that of ERK5 transcriptional targets (c-MYC and MEF2D), suggesting a link with ERK5 nuclear localization. In vitro, KPNA2 KD reduced nuclear ERK5 localization in both unstimulated and stimulated cellular models, while KD of other isoforms had no significant effect, indicating a predominant role for KPNA2. Additionally, KPNA2 KD reduced cell number in culture, supporting its role in cancer cell proliferation.

CONCLUSIONS

KPNA2 appears to be the main importin α isoform mediating ERK5 nuclear translocation. Its identification opens the possibility to develop selective inhibitors to block this process and improve ERK5-targeted melanoma therapies.

P137

UNRAVELING THE CROSSTALK BETWEEN YY1 BINDING ACTIVITY AND DNA METHYLATION IN SHAPING CANCER-ASSOCIATED PATHWAYS

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BACKGROUND-AIM

Yin Yang 1 (YY1) is a ubiquitously expressed transcription factor that plays a crucial role in regulating various biological processes under both physiological and pathological conditions. YY1 may act either as a transcriptional activator or repressor of numerous genes, most of which contain YY1 binding motifs in their promoter and enhancer regions. YY1 binding is influenced by the chromatin structure, interactions with co-activators or co-repressors, and other transcriptional modifiers, including DNA methylation. On these bases, the present study aimed to investigate the role of DNA methylation in regulating YY1 expression and binding activity in cancer.

METHODS

YY1 gene expression and DNA methylation profiling of related CG probesets were evaluated in 33 tumor types and a cohort of normal samples using TCGA datasets. YY1 ChIP-seq data of eight cell lines, retrieved from the ENCODE project, were used for peak calling and CG clustering of target sequences based on their DNA methylation status. Finally, pathway enrichment analysis was performed using STRING and Cytoscape tools to identify biological processes affected by YY1 binding and aberrant DNA methylation in cancer compared to normal cell lines.

RESULTS

Computational analyses demonstrated that YY1 is highly expressed across TCGA tumors and the normal cohort, whereas DNA methylation profiling revealed that all YY1-associated CG probesets were strongly hypomethylated, except for those mapped to the 3'UTR that were hypermethylated. Correlation analysis between YY1 expression and DNA methylation showed strong negative correlation pairs in DLBC and OV. ChIP-seq data analysis indicated that YY1 binding was positively influenced by DNA hypomethylation of YY1 consensus sites within gene promoter regions. Interestingly, gene ontology analysis highlighted that changes in DNA methylation status and YY1 binding significantly affected genes involved in nervous system development in SKNSH cell line and the homophilic cell adhesion pathway in K562 and HepG2 cells.

CONCLUSIONS

The interplay between YY1 binding and DNA methylation status of associated CG clusters suggests that targeting these CG hotspots could pave the way for the development of effective epigenetic-based therapeutic strategies against cancer.

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ANTITUMOR EFFECTS OF EXTRACELLULAR VESICLES DERIVED FROM B7-H3 CAR-T CELLS IN PANCREATIC CANCER CELLS

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BACKGROUND-AIM

Pancreatic cancer (PC) is characterized by poor prognosis and resistance to conventional immunotherapies. Chimeric antigen receptor (CAR)-T cells have emerged as a promising cell-based immunotherapy, particularly in hematologic malignancies. However, their efficacy in solid tumors remains limited. Extracellular vesicles (EVs) derived from CAR-T cells represent a potential advancement in CAR-T–based therapies. In this study, we evaluated the antitumor activity of EVs derived from CAR-T cells targeting B7-H3, a protein overexpressed in PC cells and demonstrated safety in clinical trials.

METHODS

Extracellular vesicles (EVs) derived from B7-H3 CAR-T cells were isolated using a GMP-compliant magnetic bead-based method, and characterized by AFM, Western Blot, NTA, TEM, and Flow Cytometry. In vivo pharmacokinetics were assessed in female NOD/SCID mice intravenously injected with 12 × 10# EVs/mL, and plasma data were analyzed using a two-compartment model.The antitumor activity of EVs derived from B7-H3 CAR-T cells was evaluated using MTT and 7-AAD cytotoxicity assays on PC cell lines expressing different levels of B7-H3.

RESULTS

EVs derived from B7-H3 CAR-T cells displayed a globular shape and an average size of 140 nanometers. These EVs expressed ISEV canonical markers (CD63 and Flotillin-1) and were negative for Cytochrome C. Pharmacokinetic analysis estimated an EVs plasma half-life of ~15 hours. Notably, EVs derived from B7-H3 CAR-T cells exhibited time- and cell line-dependent cytotoxic activity that correlated with B7-H3 expression levels. In line with the killing assay results, EVs derived from B7H3 CAR-T cells significantly inhibited PC cell viability, although with distinct responsivness across the tested PC cell lines.

CONCLUSIONS

Our results demonstrated that EVs derived from B7-H3 CAR-T cells exert significant antitumor effects against PC cell lines and display favorable pharmacokinetic properties. Ongoing in vivo studies will further assess their therapeutic potential.

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INSULIN-INDUCED S100A8/A9/RAGE SIGNALING PROMPTS ONCOGENIC RESPONSES IN BREAST CANCER

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BACKGROUND-AIM

Worse prognosis is observed in luminal A breast cancer (BC) patients affected by obesity[1]. In this condition, the hyperactivation of the insulin/IGF signaling (IIGFs) drives the acquisition of malignant features[2].

Calprotectin, a heterodimeric complex formed by the Ca^{2+}binding proteins S100A8 and S100A9, triggers oncogenic responses through its binding to RAGE (Receptor for Advanced Glycation End Products), a multi-ligand receptor that contributes to the complications of obesity[3-5]. Herein, we sought to evaluate the role of the Calprotectin/RAGE axis in BC progression in insulin-rich environments.

METHODS

The publicly available TCGA dataset[6] was analyzed to correlate the expression of S100A8 and S100A9 with clinical outcomes in BC patients. MCF7 BC cells, which recapitulate the molecular features of luminal A BC, were used to evaluate insulin-induced S100A8/A9 expression and the pathways involved. To this aim, pharmacological inhibitors and gene silencing strategies were employed. MCF7 cells manipulated for (i) the deletion of the insulin receptor (IR) via CRISPR/Cas9 (ii) the overexpression of the IR ligand IGF2 via lentiviral transduction were generated. These model systems were interrogated using molecular and biological studies to get further insight into the role of the S100A8/A9/RAGE signaling in insulin-induced oncogenic responses.

RESULTS

S100A8 and S100A9 gene expression correlated with worse overall- and disease-specific survival in BC patients. In MCF7 BC cells, insulin induced the upregulation of both S100A8 and S100A9 at the gene and protein levels through the IR/IRS1/ERK/AKT transduction pathway. The transcription factor HIF-1α was implicated in S100A8/A9 increase by insulin. In a model of autocrine IGF2 signaling, the overexpression of IGF2 was sufficient to support HIF-1α-mediated S100A8/A9 increase. Biologically, the increased colony-forming ability observed in IGF2-overexpressing BC cells was suppressed by the pharmacological inhibition of RAGE.

CONCLUSIONS

S100A8/A9/RAGE axis represents a novel target to halt insulin-induced oncogenic signaling. Further studies are warranted to better dissect the usefulness of anti-S100A8/A9/RAGE strategies in BC patients with de-regulated IIGFs.

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INVESTIGATING THE ROLE OF PTX3 IN THE BIOLOGY OF GLIOBLASTOMA

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BACKGROUND-AIM

Glioblastoma Multiforme (GBM) is the most common and aggressive brain tumor in adults. Despite therapies, GBM relapses are inevitable, mainly due to a subpopulation of stem cells, called glioblastoma stem cells (GSC), localized in specialized niches and resistant to conventional treatments. Pentraxin 3 (PTX3) is a soluble pattern recognition receptor belonging to the humoral arm of the innate immunity that is also involved in several aspects of tumor progression. To date, a correlation between PTX3 and tumor aggressiveness in GBM has been described, but studies regarding its role in GSC are still missing, as well as the identification of the biological pathways involved. In order to investigate this issue, we used different in vitro and in vivo models, increasing the complexity system and moving closer to the pathological scenario found in humans.

METHODS

We used human GSC BT302 cells to obtain PTX3 silenced cells. PTX3 presence and production was assessed by Western blot, qPCR, ELISA and immunostainings. We analysed the cell culture features and we generated organoids as in vitro models, while we performed subcutaneous and orthotopic injection in immunodeficient mice to evaluate the effect in PTX3 knockdown in vivo. Proliferation, invasion and angiogenic assays were performed to analyse the effects of PTX3 silencing. In addition, RNA microarray analysis was conducted in order to identify the up- and down-regulated pathways, in response to PTX3 modulation. According to the output of the bioinformatic analysis, a further characterization through immunohistochemical stainings has been carried out.

RESULTS

Preliminary observations revealed a wide expression of PTX3 in GSC, and specific silencing in a GSC line revealed a significant reduction of cell growth, invasiveness and angiogenic capacity in GSC after PTX3 knock-down. In vivo this resulted in decreased tumorigenic capacity. Furthermore, bioinformatic analysis revealed a modulation of apoptotic and hypoxia-related pathways, whose activation seems to be altered after PTX3 silencing. Accordingly, immunohistochemical characterization revealed an effective increase in hypoxia levels and a decrease of the proliferation rate in the PTX3 silenced conditions.

CONCLUSIONS

Our data suggest that PTX3 is expressed and may play a relevant role in GSC cells. In this context, PTX3 silencing may impair tumor features in vitro and in vivo. These data sets the basis for further characterization of the pro-tumoral role of PTX3 in GBM.

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ROLE OF AMBRA1 IN THE AUTOPHAGOSOME FORMATION AT RAFT-LIKE NE/ER CONTACT SITES DURING AUTOPHAGY

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BACKGROUND-AIM

In eukaryotic cells organelles are physically connected via membrane contact sites (MCS), allowing intracellular communication and functional integration of cellular processes. MCS can also directly regulate the growing of phagophore and mature autophagosome. We previously demonstrated that, upon autophagy induction, AMBRA1 is recruited to the BECN1 complex and relocalizes to mitochondria-ER contact sites, where it regulates autophagy by interacting with raft-like components. Notably proteins, such as LMNB1 and LC3-II, are implicated in the formation of nuclear autophagosomes, indicating an interplay between nuclear structures and the autophagic machinery. Since nucleophagy contributes to nuclear stability and the DNA damage response, our study aimed to elucidate molecular mechanisms involving lipid raft components.

METHODS

We isolated enriched nuclear fractions from human osteosarcoma U2OS cells under normal and upon nutrient starvation. AMBRA1 immunoprecipitates were analyzed using antibodies against LC3-II, ERLIN1 and LMNB1 to identify associated autophagosome biogenesis related proteins at nuclear envelope/ER (NE/ER) contact sites. GD3 and AMBRA1 localization was also evaluated through confocal microscopy analysis.

RESULTS

In autophagy-stimulated cells, we observed increased nuclear levels of AMBRA1, LC3-II, and ER lipid raft protein ERLIN1, suggesting their recruitment to NE/ER microdomains. Co-immunoprecipitation revealed the interaction between AMBRA1, LC3-II and ERLIN1 within GD3-positive raft-like microdomains at NE/ER contact sites. Additionally, we identified a potential interaction between AMBRA1 and tubulin suggesting a microtubule-dependent translocation mechanism to the NE/ER microdomains.

CONCLUSIONS

Our findings identified AMBRA1, LC3-II, ERLIN1, and GD3 as key players at raft-like NE/ER contact sites. This indicates a role for these domains in organizing autophagic components during nuclear autophagy. Moreover, a potential microtubule-based mechanism for an alternative AMBRA1 translocation to the nucleus, contributing to autophagosome assembly and nuclear material degradation. This provides a basis for future studies on the molecular regulation of nucleophagy by raft-like NE/ER contact sites and its role in disease conditions.

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THE ROLE OF HYPOXIA-DRIVEN LIPID METABOLISM IN MODULATING ANGIOGENESIS IN GASTROINTESTINAL MALIGNANCIES

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BACKGROUND-AIM

Gastrointestinal cancers, including gastric, pancreatic, and colorectal cancers, are heterogeneous diseases with different etiologies and clinical management. In this complex scenario, oxygen limitation is a common condition that fosters cancer malignancy by stimulating glycolysis and inhibiting mitochondrial activity. In particular, hypoxia can reprogram fatty acids (FA) and lipid metabolism, enhancing FA synthesis and uptake, which in turn affect tumor angiogenesis, a relationship that deserves further investigation.

METHODS

Nine cancer cell lines (gastric, pancreatic, and colon) were subjected to hypoxia for 24 or 72 hours as acute or chronic stimuli. Mitochondrial mass and membrane potential were analyzed by flow cytometry. Metabolic profiling was performed using the Seahorse platform, while intracellular lipids were detected with BODIPY 493/503 staining. Lipid secretion produced by cancer cells was measured via Nile Red, and human endothelial cells subjected to such conditioned media (CM) were assayed via 3D models for their angiogenic capacity.

RESULTS

A chronic hypoxic stimulus was capable of inducing an increased FA storage, as intracellular neutral lipids, which is often paired by an enhanced lipid secretion. We observed that CM could regulate vessel formation in an inversely proportional manner to the amount of lipids contained. We validated this negative tendency by administering increasing doses of palmitic acid. Paradoxically, lipid-rich CM increased endothelial cells maximal respiration, a key function for angiogenesis, suggesting a more complex metabolic interaction.

CONCLUSIONS

Our findings suggest that chronic hypoxia induces a metabolic shift in gastrointestinal cancer cells, increasing FA storage and secretion, which in turn modulates endothelial cell behavior and angiogenesis. The inverse correlation between secreted FA levels and vessel formation highlights a potential mechanism for aberrant tumor vascularization. Interestingly, gastric cancer cells appear to follow a distinct metabolic pattern, warranting further investigation. Overall, these findings highlight how prolonged hypoxia marks a metabolic turning point, offering novel therapeutic avenues to target tumor angiogenesis via FA metabolism.

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FROM RADIATION TO IMMUNE ACTIVATION: OPTIMIZING RADIOIMMUNOTHERAPY SYNERGY IN SOLID TUMORS

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BACKGROUND-AIM

Immunotherapy has transformed the treatment landscape for solid tumors, yet resistance remains a major challenge. Radiotherapy (RT) has emerged as a promising approach to modulate the tumor microenvironment, enhance antigen presentation, and boost adaptive antitumor immunity, potentially improving immunotherapy outcomes. Beyond its local effects, RT can induce the abscopal effect, where localized treatment leads to the regression of distant lesions via immune activation. RT induces the release of damage-associated molecular patterns (DAMPs), collectively contributing to systemic anti-cancer immune responses. Understanding DAMPs dynamics is crucial to optimize RT-immunotherapy combinations, particularly regarding dosing and scheduling. This study aims to optimize RT protocols to maximize tumor immunogenicity and anti-cancer immune activation.

METHODS

Tumor cell lines of various origins, including cutaneous melanoma, glioblastoma, neuroblastoma, osteosarcoma, breast, gastric, pancreatic, renal, and lung carcinomas, were cultured in 2D and 3D systems and treated with RT doses from 2 to 6 Gy. Cultures were analyzed at multiple time points (up to 72h for 2D, 7 days for 3D) for DAMP exposure/release (calreticulin, HMGB1) by flow cytometry and ELISA. PD-L1 expression was also assessed by flow cytometry. Co-cultures with PBMCs were used to evaluate immune activation, cytotoxicity (granzyme B, perforin), and tumor cell death (spheroid volume, caspase-3 staining).

RESULTS

Across the various tumor types tested, we observed consistent increases in DAMP release and signs of immune activation following RT. Specifically, membrane calreticulin exposure and HMGB1 release increased in a time- and dose-related manner. PD-L1 upregulation was also observed under the same conditions. In tumor spheroids exposed to 2, 4, or 6 Gy, the caspase-3 signal was stronger when co-cultured with PBMCs, suggesting enhanced immune-mediated tumor cell death. Supporting this, PBMCs co-cultured with RT-treated tumor cells showed increased perforin and granzyme B expression, indicating greater cytotoxic capacity.

CONCLUSIONS

These findings highlight the immunogenic potential of RT and its ability to enhance immune-mediated tumor killing, supporting the rationale for optimized radioimmunotherapy strategies in solid tumors.

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EFFECT OF 5ALA-PDT ON CELL PROLIFERATION, APOPTOSIS, AND INVASION IN OSCC CELL LINES

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BACKGROUND-AIM

Oral squamous cell carcinoma (OSCC) is a highly aggressive malignancy that develops in the epithelial cells of the head and neck region. OSCC constitutes more than 90% of all oral cancers. The high recurrence rate makes these challenging targets for therapies. 5-aminolevulinic acid (5ALA) leads to the accumulation of protoporphyrin IX (PpIX), a molecule in the heme biosynthetic pathway. Photodynamic Therapy (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). Administration of exogenous 5-ALA improves the production of PpIX by cancer cells, increasing the rates of cell death. This study analyzes the effect of ALAD-PDT on OSCC cell lines.

METHODS

In this study, low doses of 5ALA were applied to CAL27 and OECM1 cells for 8 hours, followed by exposure to a 630nm LED device. Cytotoxic effects were measured using the MTS test. On both cell lines, flow cytometry and Annexin-V staining were performed to assess the influence of 5ALA-PDT on the apoptosis and the cell cycle. Gene expression of apoptosis and ROS was analyzed by qRT-PCR.

RESULTS

The highest cell mortality rate of cell line was obtained at 1.84 mM for 8 hours. The apoptosis assay confirmed the results of MTS. Cell cycle alterations were observed. The flow cytometric analysis with PI staining showed that 5ALA-PDT induced a marked cell cycle variation, resulting in the arrest of a large percentage of cancer cells in the S/G2 phase. Regarding qPCR of ROS genes, the expression of SOD and CAT genes increases while the expression of iNOS is still unclear. Regarding qPCR of apoptosis genes, BAX and Bcl-2 expressions increase. The expression of p21 has a linear trend, which increases with increasing 5ALA concentrations.

CONCLUSIONS

ALAD-PDT markedly inhibited the viability of OSCC cell lines through mechanisms involving the accumulation of PpIX that, under irradiation, can induce profound ROS production and phototoxicity. This represents a premise for obtaining new therapeutic protocols aimed at improving the clinical outcome of patients affected by OSCC. The use of a combined polypharmacological treatment would be desirable to increase prognosis, survival and prevent the development of resistance in particularly aggressive tumors.

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MOLECULAR DIAGNOSIS OF HEREDITARY HEMOCROMATOSIS BY NEXT GENERATION SEQUENCING: A CASE REPORT

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BACKGROUND-AIM

Hereditary hemochromatosis (HH) is the most common genetic disease associated with iron metabolism and generally manifests in adulthood (fifth-sixth decade of life), when the effects of iron overload in hepatocytes and cardiac cells become apparent.
Many genes are involved in the pathogenesis of HH, but the most frequent is the HFE gene. Several rarer forms of HH have also been characterized molecularly, including those secondary to mutations in hepcidin, hemojuvelin, transferrin receptor, and ferroportin genes. HH unrelated to HFE, however, is rare.
The prognosis for affected patients depends on the timeliness of diagnosis. If diagnosis occurs early and treatment (phlebotomy) is initiated before significant liver and heart damage, patients can expect to live a normal lifespan. In this context, Next Generation Sequencing (NGS) platforms are of considerable importance: it is crucial for confirming HH diagnosis and identifying less frequent mutations implicated in this pathology, that do not involve HFE gene.

METHODS

We present a clinical case that highlights the importance of NGS in diagnosing HH. The case involves a completely asymptomatic 27-year-old patient who presented with an enlarged liver and steatosis, as well as markedly increased ferritin and transferrin saturation, and hepatocyte indices at the upper limit of the reference range. We performed genetic analysis of HH, considering not only the HFE gene but the completed exome, by NGS.

RESULTS

To confirm diagnosis of HH, NGS was performed and showed the presence of HFE:c.845G>A- p.Cys282Tyr (rs1800562) in homozygosity. NGS analysis showed also other mutations in genes implicated with HH and iron overload, like TFR2:c.1473G>A-p.Glu491Glu (rs139178017) and PIEZO1:c.3602C>T-p.Thr1201Met (rs372935580), both in heterozygosity. An unexpected mutation SPTA1:c.5572C>G-p.Leu1858Val (rs3737515), in heterozygosity, common in patients with spherocytosis, was also identified.

CONCLUSIONS

NGS was used to confirm the diagnosis of HH and identify an unexpected defect in the red blood cell membrane, which was also highlighted by further laboratory tests (haptoglobin and peripheral blood smear). Genetic studies based on accurate NGS platforms could therefore characterise patients' conditions and their therapeutic management more effectively.

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OLIVE PATÉ: A VALUABLE BYPRODUCT WITH ANTI-INFLAMMATORY PROPERTIES

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BACKGROUND-AIM

The growing interest in the circular economy and the valorization of agri-food waste has led to the exploration of the nutraceutical properties of various naturally derived compounds. Polyphenols are widely present in plants and exhibit several biological effects, including anti-inflammatory properties, which make them particularly suitable for therapeutic use. In this study we focused on olive paté, a compound rich in polyphenols (mainly verbascoside and hydroxytyrosol) with potential anti-inflammatory effects.

METHODS

The anti-inflammatory effect was evaluated on murine macrophages (RAW264.7). The cells were treated for 24 hours with lipopolysaccharide (LPS) to induce inflammation and/or olive paté (P7). Cytotoxicity was assessed using MTT assay; nitric oxide production was measured with the Griess assay; and the protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) was analyzed with Western Blot. The same analyses were conducted using standard verbascoside and hydroxytyrosol or a combination of both, at concentrations found in P7.

RESULTS

P7 showed cytotoxicity only at doses above 200 μg/mL after 24 hours of treatment; therefore, doses of 10, 50 and 100 μg/mL were selected for the subsequent analyses. Following inflammatory stimulation with LPS, treatment of P7 resulted in a dose-dependent reduction of nitric oxide production. The expression of iNOS (a key enzyme in the macrophage inflammatory response) was significantly reduced at all doses, while COX2 (a pro-inflammatory enzyme) was significantly reduced only at the 100 μg/mL dose. Verbascoside did not reduce nitric oxide production, while hydroxytyrosol and the combination of the two standards were effective only at the highest dose.

CONCLUSIONS

Olive paté demonstrated effective anti-inflammatory activity, but these effects can only be partially attributed to its content of verbascoside and hydroxytyrosol, the main polyphenols in paté. Therefore, olive paté represents a promising waste-derived product for potential pharmacological or nutraceutical applications. Further investigation is needed to identify the active molecules responsible for this effect and to elucidate the underlying mechanisms of action.

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INVOLVEMENT OF INNATE LYMPHOCYTES IN THE PATHOGENIC MECHANISMS OF COLORECTAL CANCER

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BACKGROUND-AIM

Colorectal cancer (CRC) is one of the most common types of cancer globally. Studying the complex immunological microenvironment of CRC patients is crucial both for understanding the immune circuits involved in progression and control, and for assessing the response to immunotherapeutic treatments.
In this study we have focused our attention on innate lymphoid cells (ILC) within the tumor microenvironment, which constitute the "unconventional" lymphocytes of the immune system.
A deeper understanding of the role of these subsets could offer advantages for immunotherapeutic approaches and for improving treatments of patients.

METHODS

Flow cytometry analysis of isolated cells has provided a detailed view of phenotypic characteristics, allowing for an in-depth evaluation of surface marker expression. Therefore, we characterized CRC-infiltrating innate immune cells to better understand the complexity of the immune response to tumors. Specifically, in a heterogeneous cohort of 11 patients, we assessed the frequency of ILC subsets by flow cytometry in peripheral blood, healthy tissue, and tumor tissue. PBMCs were isolated from peripheral blood using Ficoll gradient centrifugation, while tumor-infiltrating lymphocytes (TILs) were obtained from tissue biopsies through mechanical and enzymatic digestion, enabling the isolation of target cell populations.
Immunophenotypic analysis for ILCs was performed based on the expression of the following surface markers: CD127, CD117, CRTH2, CD45, and lineage-negative (Lin−) markers.

RESULTS

The immuno-phenotypic analysis showed heterogeneous distribution of ILC2 and ILC3 subsets in healthy mucosa. In peripheral blood, all three subsets (ILC1, ILC2, ILC3) were uniformly present across patients but exhibited individual variability, suggesting no consistent frequency patterns. In tumor-associated tissue, ILC1 and ILC3 showed uniform distribution without dominant values, while ILC2 were absent, indicating a potential common feature linked to tumor.

CONCLUSIONS

These results highlight the complex and compartment-specific behavior of ILC populations in CRC patients and may provide insights into their potential role in tumor immunity. Future developments of this study will include functional assays to evaluate the proliferative capacity, cytotoxic activity, and cytokine production of ILC populations, aiming to better elucidate immune dynamics in CRC and their clinical implications.

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DIFFERENTIALLY EXPRESSED MICRORNAS TARGETING THE DREAM COMPLEX AND THE CDK INHIBITOR ABEMACICLIB: POTENTIAL FOR TRANSLATION AGAINST HEAD AND NECK CANCERS

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BACKGROUND-AIM

Head and neck squamous cell carcinomas (HNSCC) are characterized by rapid progression and poor survival. The multiprotein Dimerization partner, RB-like, E2F, And Multi-vulval class B (DREAM) complex maintains cells in quiescence by recruiting RB-like and E2F co-repressors to the MuvB core. CDK activation displaces these repressors, allowing BMYB and/or FOXM1 to bind the MuvB core and activate proliferation genes. FOXM1 maintains high proliferative potential in keratinocytes and acts as a driver of laryngeal squamous carcinoma.
We aimed to identify miRNAs regulating the DREAM and BMYB/FOXM1-MuvB complexes in HNSCCs, characterize their effects on tumorigenic features and evaluate the potential synergy between miRNA targeting strategies along with CDK inhibitors (such as abemaciclib) to counteract cancer progression.

METHODS

TaqMan Array Card-A was used to study miRNA expression in laryngeal cancer specimens. In silico analyses were performed to select miRNAs predicted to target DREAM/BMYB/FOXM1-MuvB complex components, followed by quantitative real-time PCR (qRT-PCR) on HNSCC cell lines. Simultaneously, the effects of abemaciclib were assessed by WST-8, clonogenic assays, FACS analysis and western blotting.

RESULTS

A signature of differentially expressed miRNAs were identified in laryngeal carcinomas compared to adjacent normal tissues. Among these, miR186-5 and miR204-5p were downregulated whereas miR20a-5p; miR106a-5p miR17-5p were upregulated and all were predicted to target members of the DREAM/BMYB-FOXM1 complexes, through in silico analyses. QRT-PCR analysis confirmed miRNA expression levels in different HNSCC cell lines. In parallel, the CDK4/6 inhibitor, abemaciclib proved able to reduce cancer cell viability of HNO210, HEp2 and FaDu cells without showing toxicity on the immortalized human keratinocyte cell line HaCaT. Abemaciclib also inhibited cancer cell colony forming ability. FACS analyses showed cell accumulation in G1 or G2 cell cycle phases upon 48 and 72h treatment with abemaciclib at the IC25 and IC50, respectively. Consistently, an increase in the expression of the cell cycle inhibitor p21 was observed, likely independent from p53. This was paralleled by a decrease in AKT activation.

CONCLUSIONS

These results support the study of cell cycle regulation mechanisms in HNSCC, evaluating the potential role of targeting deregulated miRNAs that affect the DREAM function in synergy with the new clinically approved CDK4/6 inhibitor abemaciclib.

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PHENOTYPIC CHARACTERIZATION OF PORCINE KIDNEY-RESIDENT NK CELLS AND MONONUCLEAR LEUKOCYTES: COMPARISON WITH AUTOLOGOUS BLOOD

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BACKGROUND-AIM

The porcine kidney is increasingly recognized as a valuable model in both translational immunology and regenerative research. In addition to its emerging role in xenotransplantation, it may represent a relevant platform for studying immune cell dynamics in tissue homeostasis and injury. In this context, a better understanding of local immune cell subsets—including tissue-resident Natural Killer (NK) cells—is critical to assess immunogenicity and inflammatory potential. While porcine liver NK cells have been partially characterized, data on renal NK cells remain scarce. Our aim was to profile NK cells and mononuclear leukocytes in normal porcine kidney tissue and compare them to circulating populations from autologous blood. To analyze the possible interactions involving immune cells in healthy kidney, culture of expanded renal cells were phenotypically analyzed.

METHODS

Mononuclear cells were isolated from fresh kidney samples by enzymatic digestion and from peripheral blood (PB) by density gradient separation. Flow cytometry was used to assess the expression of CD8α, NKp46, and T-bet to delineate NK subsets. CD3ε and CD172a were included to recognize T subsets and myeloid cells. Renal epithelial markers were evaluated to confirm the presence of non-immune components.

RESULTS

Preliminary analyses showed an increased frequency of CD8α^+ CD3ε^- CD172a$^-$ lymphocytes in the kidney compared to PB (mean 37.6% vs 15.7%), consistent with reported NK cell proportions in human renal tissue. Further phenotypic profiling revealed a higher frequency of NKp46$^+$ T-bet$^+$ cells among total lymphocytes in the kidney (mean 33.6%) versus blood (mean 13.4%), suggestive of an enrichment of tissue-associated NK cells with resident-like features. Kidney cell suspensions also included variable proportions of resident CD3ε^+ lymphocytes, myeloid populations and viable epithelial cells. Phenotypic analysis of cultured renal cells showed the expression of ligands specific for NK cell receptors.

CONCLUSIONS

These results support the existence of a distinct tissue-associated NK cell subset in the porcine kidney. This foundational dataset contributes to the immunological mapping of porcine kidneys and may inform future strategies in immunopathology, tissue engineering, and xenotransplantation.

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INTEGRATED MULTI-OMICS ANALYSIS OF IMMUNE AND MICROBIAL DYSREGULATION IN THE EVOLUTION OF MULTIPLE MYELOMA

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BACKGROUND-AIM

Multiple myeloma (MM) is an incurable hematologic malignancy characterized by the clonal expansion of plasma cells in the bone marrow (BM). It is typically preceded by asymptomatic stages: monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). Although only 5–10% of MGUS cases progress to MM annually, the underlying mechanisms remain poorly defined. Emerging evidence suggests that immune dysregulation and microbiota alterations may drive disease evolution.

METHODS

To explore this, we applied an integrated multi-omics strategy combining single-cell transcriptomics, immunophenotyping, soluble factor profiling, and microbiome analysis. A total of 106 patients across the MGUS–SMM–MM spectrum were enrolled, with paired BM, peripheral blood, and stool samples collected. Single-cell RNA sequencing and flow cytometry characterized immune landscapes. Cytokine and chemokine levels were measured via 48-plex Luminex assay in 72 patients and 4 healthy donors (HDs). Gut microbiome profiling was performed on 41 fecal samples (10 MGUS, 15 SMM, 16 MM).

RESULTS

As the disease progresses from MGUS to MM, we observe a progressive increase in memory and naïve B cells, unconventional T cells (including MAIT and $\gamma\delta$ T cells), and CD8 effector and memory T cells in MM. This transition isalso marked by a progressive expansion of clonal T cell populations, particularly among CD8 effector/memory and unconventional T cells. However, despite this clonal expansion, surface protein analysis reveals hallmarks of T cell exhaustion and senescence—most notably, increased CD57 and reduced expression of ICOS, CD27, CD127, and CD161—indicating a functional decline and reduced anti-tumor capacity. Flow cytometry further confirmed the expansion of CD8 effectors and a predominance of exhausted/effector $\gamma\delta$ T cells in MM. BM cytokine profiling showed reduced levels of GM-CSF, IFN-α2, IFN-γ, IL-1β, IL-2, IL-2Rα, IL-3, IL-10, IL-13, LIF, and MCP-1/CCL2 in MM vs MGUS/SMM. Microbiota analysis showed enrichment of anti-inflammatory taxa (Lachnospirales, Oscillospirales, Ruminococcaceae, Bifidobacteriaceae) in MGUS, while SMM/MM samples displayed a pro-inflammatory shift (Bacteroidaceae, Enterobacteriaceae, Streptococcaceae).

CONCLUSIONS

These data support immune and microbial signatures as potential predictive biomarkers and therapeutic targets in MM progression.

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ANALYSIS OF T CELL SUBSETS IN PERIPHERAL BLOOD OF MULTIPLE SCLEROSIS PATIENTS TREATED WITH ANTI-CD20 MAB THERAPY

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BACKGROUND-AIM

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, and neurodegeneration. Both B and T lymphocytes are crucial in the disease process. Anti-CD20 monoclonal antibody therapies, targeting CD20-expressing B cells, have proven clinically very effective in suppressing MS activity and progression, due to modification of immune responses.
The aim of the study is to assess the frequency and phenotype of T cell populations in the peripheral blood of MS patients undergoing anti-CD20 therapy to understand the long-term influence of these treatments on immune system cells and their relationship with disease activity and progression.

METHODS

Peripheral blood samples from MS patients were analyzed before and after anti-CD20 treatment (rituximab, ocrelizumab, ofatumumab) using advanced flow cytometry techniques.

RESULTS

CD4+ and CD8+ T cells demonstrated an enhanced frequency of naive and regulatory subsets and reduced frequencies of effector memory subsets. More surprisingly, $\gamma\delta$ T cells, traditionally considered innate-like immune cells, also underwent substantial redistribution across naive and memory compartments, supporting a broader immunomodulatory effect of the therapy.

CONCLUSIONS

These preliminary results highlight the complexity of immune alterations induced by anti-CD20 therapy in MS patients and could support the need to develop personalized treatment approaches to optimize the long-term outcomes for MS patients.

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DISSECTING THE FUNCTIONAL ROLE OF THE ONCOGENIC LNCRNA PVT1 AS A NOVEL PUTATIVE MOLECULAR TARGET FOR BREAST CANCER TREATMENT

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BACKGROUND-AIM

The occurrence of resistance mechanisms to endocrine therapies represents a relevant problem in the clinical management of Estrogen Receptor alpha (ERα) positive breast cancers (BCs). To overcome this issue, the dissection of ERα regulatory machinery and the characterization of its functions as an RNA binding protein (RBP) represent innovative approaches to find novel putative molecular targets against hormone responsive BCs. Among ERα molecular partners, we focused our attention on a subset of fitness ERα-interacting lncRNAs. Previous studies from our group demonstrated the oncogenic role of the ERα-interacting lncRNA PVT1, which can modulate the formation of multi-molecular complexes to regulate the expression of specific tumor-promoting genes in MCF-7 BC cell models.

METHODS

We adopted ASO-mediated lncRNA gene silencing to investigate the role of PVT1 in both responsive and anti-estrogen resistant cell models. Furthermore, we applied an integrative omics and molecular approach to gain deeper insights into its involvement in the regulation of gene expression.

RESULTS

The essential ERα-interacting lncRNA PVT1 was selected for functional investigations due to its oncogenic functions, its overexpression in BC samples and our previous results. Hi-C and mass spectrometry-based experiments provided the milestones for characterization of ERα-PVT1 chromatin associated machinery. Moreover, loss-of-function assays displayed promising results across different BC cellular systems. Particularly, the transcriptome profiling of ERα positive, ERα negative and anti-estrogen resistant cell models suggested a potential involvement of PVT1 in different cancer-promoting pathways.

CONCLUSIONS

The obtained results reveal that PVT1 is a core molecule in the modulation of tumor suppressor genes in multiple BC models, suggesting its potential as a novel molecular target to interfere with ERα-depending BC progression.

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DECODING THE COMPLEXITY OF SéZARY SYNDROME THROUGH NEXT-GENERATION MULTI-OMICS APPROACHES

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BACKGROUND-AIM

Sézary syndrome (SS) is a rare, aggressive form of cutaneous T-cell lymphoma originating from malignant peripheral CD4+ T-cells that can infiltrate the skin, blood, and lymph nodes, causing widespread symptoms. It is a chronic and incurable disease, and it is associated with poor prognosis and significant impairment of life quality. Despite next-generation sequencing (NGS)-driven insights, its biology remains unclear mainly due to tumor heterogeneity. To address these challenges, the integration of NGS-based multi-omics approaches holds promise for unraveling the complex biological mechanisms underlying SS.

METHODS

Whole-genome sequencing (WGS) was conducted on SS cell lines to explore DNA alterations with the aim to identify potential cancer-driving mutations. In parallel, transcriptomic analysis was performed on SS patient-derived cell cultures and compared to healthy donors (HD). Specifically, RNA was extracted from CD4+ malignant lymphocytes and from CD4+ cells of HD. Additionally, DNA from a subset of these patients and healthy donors was analyzed for methylation patterns using a microarray-based methylome profiling.

RESULTS

Globally, WGS analysis on SS lines revealed that several mitochondrial genes appear to be affected. The patient cohort includes both treatment-naïve individuals (T1) and (T2) pre-treated patients with 10 cycles of extracorporeal photopheresis and interferon-α. Transcriptomic analysis identified 655 differentially expressed genes (DEGs) when comparing SS patients with HD and 47 when comparing T1 and T2 patients. Following treatment, several pathways related to key cancer hallmarks, such as oxidative phosphorylation, MYC and mTOR signaling, and DNA repair, were modulated. Among the upregulated genes in SS patients, KIR3DL2, KCNN4, TWIST1, RAB37, C4orf48, and CCDC167 demonstrated significant prognostic value and represent potential novel biomarkers. These transcriptomic findings were further supported by methylome analysis conducted on the same patient samples.

CONCLUSIONS

Our integrative multi-omics analysis highlights critical molecular alterations in Sézary syndrome, combining genomic, transcriptomic, and epigenetic data to unravel the complexity of SS and improve clinical outcomes.

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INTEGRATIVE MULTI-OMICS ANALYSIS UNCOVERS MOLECULAR MECHANISMS IN CARDIAC MYXOMA

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BACKGROUND-AIM

Cardiac myxoma (CM) is the most common primary tumour of the human heart. Despite CM is a benign neoplasm and often asymptomatic, its intracavitary growth hampers the regular blood flow, leading to miscellaneous severe complications, including embolism, ischemia, myocardial infarction, or cardiac arrest. CM presents heterogeneous clinical manifestations, with approximately ~90% of sporadic cases, while familial forms are associated with Carney Complex and germinal mutations in PRKAR1A gene. Currently, surgical resection is the only resolutive treatment, and the development of new pharmacological treatments is a current task. This study aim to investigate the aetiology and molecular pathogenesis of CM using an integrative multi-omics approach.

METHODS

Formalin-fixed Paraffin-Embedded tissues from 27 CM patients were analysed using high throughput technologies. In 6 cases, adjacent not tumour tissue was also available for comparison. In particular, Exome sequencing, CpG DNA methylation arrays, RNA-sequencing and small non-coding RNA (sncRNA) sequencing were performed on this cohort. A comprehensive multi-omics strategy was employed to integrate these data and to uncover molecular mechanisms underlying CM pathogenesis.

RESULTS

Comparative analysis of gene expression, DNA methylation, and sncRNA profiles revealed distinct molecular signatures differentiating tumoral and normal tissues. However, differential analysis did not clearly segregate PRKAR1A-mutated and wild-type cases. Nonetheless, the integrative multi-omics approach, based on PLS-DA and cross-variable correlation, and deconvolution analysis, identified molecular patterns capable of discriminating not only tumoral and normal samples but also those with differing PRKAR1A mutational status. Integrative analysis revealed a network of interconnected CpG sites, transcripts, and sncRNAs associated with biological processes such as cell proliferation and tissue remodelling, highlighting coordinated regulatory mechanisms in CM pathogenesis.

CONCLUSIONS

These findings reveal the intricate molecular and functional landscape of CMs, identifying novel biological players and potential disease biomarkers that merit further investigation and may contribute to the development of future therapeutic strategies. Work was supported by AIRC (Grant IG-23068) to AW, University of Salerno (FARB to GN, FR, RT and AW), Ministry of University and Research (PRIN2022 2022Y79PT4 to AW; 20223MMYTB to FR; 202282CMEA to GN, 2022A7HJEM to RT)

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STUDY OF SOLUBLE P2X7R AS A USEFUL BIOMARKER IN ALZHEIMER'S DISEASE.

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BACKGROUND-AIM

Alzheimer's disease (AD) is a neurodegenerative and neuroinflammatory responsible of 60–70% of dementia cases. Besides the current invasive method for obtaining cerebrospinal fluid samples or the costly magnetic positron emission tomography (PET), it is essential to develop less invasive and more cost-efficient diagnostic methods. Various blood biomarkers such as Aβ42/Aβ40, neurofilaments, glial fibrillary acidic protein, t-tau and p-tau proteins, have been recently proposed.
Based on our recent studies showing that a soluble circulating form of P2X7R (sP2X7R), an ATP-gated ionotropic receptor highly expressed in innate immune cells, such as microglia, is associated with inflammatory markers (e.g. CRP) and with pathological conditions, such as COVID-19, we suggested sP2X7R as a promising candidate for AD early diagnosis.
The aim of this study was to assess the sP2X7R plasma levels in a population of AD patients, compared to age-matched healthy control (HC) subjects.
To improve diagnostic capability, and to monitor possible disease progress, we extended the study to a group of patients characterized by mild cognitive impairment (MCI).

METHODS

We assayed the plasma levels of sP2X7R by ELISA (Cusabio, Houston, USA) in a cohort of 126 subjects, including HC, MCI and AD individuals with balanced male and female distribution.

RESULTS

No significant age difference was observed between these three groups. Analysis of total sample showed that HC present negligible difference in plasma sP2X7R levels if compared with AD patients. However, MCI subjects exhibit a significant increase of sP2X7R levels when compared with HC and AD patients.
Analysing the data by gender, we found significantly higher sP2X7R levels in males compared to females, in both the HC and MCI groups, whereas no statistically significant gender difference was noticed in AD patients.
An extended statistical analysis using ROC curve was performed to investigate the diagnostic potential of plasma sP2X7R measurement to distinguish the clinical status of AD and MCI. The results allowed us to state that the measurement of plasma sP2X7R levels was not useful to differentiate AD patients from HC subjects, but was acceptable to distinguish MCI from either HC or AD.

CONCLUSIONS

Our observations indicate that: i) plasma sP2X7R is a promising novel prognostic biomarker of MCI patients, and ii) gender differences in sP2X7R expression underline the importance of considering gender as a biological variable in future related studies.

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EXPLORING THE NON-GENOMIC ACTIONS OF THE ANDROGEN RECEPTOR IN COLORECTAL CANCER

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BACKGROUND-AIM

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide. Epidemiological studies demonstrate that men have a higher CRC incidence rate and face worse survival outcomes compared to women. This gender disparity in CRC incidence and outcomes pushed us to explore the mechanisms by which the androgen receptor (AR) may influence its pathology. Interestingly, the roles of AR in cancer extend beyond traditional hormone-dependent cancers, in fact, in recent years, researchers have identified that AR signaling is also implicated in the progression of other cancers that do not typically respond to hormonal regulation. Understanding how AR mediates the CRC aggressiveness could provide valuable insights that may help to find more effective treatment strategies.

METHODS

In this study, we used CRC-derived Caco2, LoVo, and HCT-116 cells, expressing AR at different extend. BrdU incorporation assay and the measurement of spheroid growth were used to follow cell proliferation. Biochemical approaches such as co-immunoprecipitation, immunoblot, and immunofluorescence show that the androgen treatment induces the association between AR and Filamin A and the activation of different effectors without altering the AR cell localization.

RESULTS

Our findings demonstrate that, in CRC cells, androgen treatment triggers the association between AR and Filamin A. This complex activates Rac, p70, PKCs and other proteins thereby controlling different CRC-derived cells proliferation. The antiandrogen Bicalutamide and a small peptide, Rh2025u, designed to mimic the AR sequences responsible for AR/Filamin A interaction, reverse the androgen-induced effects in all cell lines.

CONCLUSIONS

The present study emphasizes the significance of AR in CRC and suggests that variations in androgen levels may influence both onset and progression of this disease. Clarifying the role of this steroid receptor in colon cancer can pave the way to developing new screening campaigns and new specific and effective therapies

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BISPHENOL A AT THE GATE: EGFR AND TRKA MEDIATE PROLIFERATIVE EFFECTS IN COLON CANCER CELLS

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BACKGROUND-AIM

Bisphenol A (BPA) is a synthetic organic compound with well-documented xenoestrogenic activity, widely used in the production of polycarbonate plastics and epoxy resins. Due to its ability to leach into food and water, BPA is a pervasive environmental contaminant commonly detected in human biological fluids. While BPA is known to exert harmful endocrine-disrupting effects, its direct impact on colorectal cancer development remains largely unexplored, despite the gastrointestinal tract being a primary route of exposure. This study investigates the proliferative effects of BPA using human colon cancer cell lines that reflect different levels of tumour progression.

METHODS

Cell proliferation was assessed using WST-1 and BrdU incorporation assays in two colorectal cancer cell lines, Caco-2 (less aggressive) and HCT-116 (more aggressive), following BPA treatment. Key signalling pathways were analyzed by western blot. The involvement of specific receptors—Epidermal Growth Factor Receptor (EGFR) and Tropomyosin receptor kinase A (TrkA)—was evaluated using the selective inhibitors ZD1839 (Iressa) and GW441756, respectively.

RESULTS

BPA significantly enhanced proliferation in both Caco-2 (less aggressive) and HCT-116 (more aggressive) colon cancer cell lines at nanomolar concentrations, alongside the downregulation of the cell cycle inhibitor protein p27 and upregulation of cyclin A, within 18 hours of exposure. BPA also induced rapid activation of downstream signaling molecules associated with proliferation. Inhibition of TrkA and EGFR effectively blocked BPA-induced receptor phosphorylation (at Tyr490 and Tyr1068, respectively) and attenuated the downstream activation of ERK, Akt, P90RSK, and GSK-3α/β.

CONCLUSIONS

These findings suggest that BPA, even at environmentally relevant concentrations, promotes colon cancer cell proliferation through activation of EGFR and TrkA signaling pathways. Further investigation using 3D culture models and primary cells from normal and cancerous colon tissues is warranted to deepen our understanding of BPA's tumor-promoting mechanisms and to support the development of effective preventive strategies.

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UNDERSTANDING THE CONSEQUENCES OF REDUCED CNBP EXPRESSION IN DM2 PATHOGENESIS

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BACKGROUND-AIM

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 incompletely 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.

METHODS

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.

RESULTS

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 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. This autophagy activation was consistently observed in 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.

CONCLUSIONS

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 opens new avenues for mechanism-based therapeutic interventions.

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CLINICAL RISK PREDICTION OF MORTALITY IN TYPE 2 DIABETES USING EXPLAINABLE MACHINE LEARNING

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BACKGROUND-AIM

Type 2 diabetes mellitus (T2DM) is a major contributor to reduced life expectancy due to its association with diverse comorbidities and complications. Accurate mortality risk stratification can facilitate timely interventions and personalized care. We developed and validated an explainable machine learning model to predict long-term all-cause mortality in individuals with T2DM.

METHODS

A longitudinal dataset of 554 patients with T2DM and up to 16.8 years of follow-up was analyzed. Using various feature selection techniques, 19 clinical and laboratory parameters were filtered and refined. Nine machine learning algorithms were evaluated, with the extra survival trees (EST) model emerging as the best performer. Interpretability was achieved through SHAP (Shapley Additive Explanations), allowing both cohort-level and individual-level explanations.

RESULTS

The optimized EST model employed 10 features, including age, NT-proBNP, RDW-SD, creatinine, and a specific glycan marker (NA3F), achieving a Harrell's C-index of 0.776 and AUC values above 0.8 for predictions at 5, 10, and 15 years. SHAP analysis revealed age, comorbidity burden, and NT-proBNP as key predictors, and enabled transparency in individualized risk assessments.

CONCLUSIONS

Our approach combines high predictive performance with clinical interpretability, offering a valuable tool for risk assessment in T2DM care. This model holds potential to inform personalized clinical decisions by identifying patients at elevated risk for mortality well in advance.

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MIR-326 DELIVERY VIA BIOCOMPATIBLE POLYMERIC NANOPARTICLES: A NOVEL THERAPEUTIC APPROACH FOR MEDULLOBLASTOMA

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common malignant pediatric brain tumor with high biological heterogeneity. Despite treatments, survival rates remain poor with severe side effects. miRNAs are promising therapeutics due to their regulation of multiple tumor pathways, but clinical use is limited by instability and poor delivery. We developed biodegradable polycaprolactone/chitosan nanoparticles (NPs) to deliver miR-326, a tumor suppressor in MB, improving miRNA stability, cellular uptake, and controlled release. We tested their biocompatibility, delivery, and biological effects in MB cell lines and in vivo.

METHODS

NPs were formed by complexing miR-326 with chitosan and coated with polyethyleneimine for stability. In vitro, MB cell lines were assessed for uptake, viability, differentiation, apoptosis, and miRNA expression. In vivo, scramble NPs were given to mice for 5 days to evaluate toxicity.

RESULTS

NPs were rapidly taken up by MB cells, peaking at 24 hours. miR-326-NPs significantly increased intracellular miR-326 levels vs. traditional transfection. miR-326, carried by NPs in different tumoral cell lines, promoted neuronal differentiation, reduced stemness markers, and induced apoptosis. Control NPs showed no toxicity in mice, with normal liver/kidney function and no tissue damage.

CONCLUSIONS

PCL/chitosan NPs effectively deliver miR-326, enhancing differentiation and apoptosis while reducing stemness in MB cells. They offer greater miRNA stability and biocompatibility than standard methods, with safe preliminary in vivo results. The platform can co-deliver drugs or biomolecules for multi-therapy and cross the blood-brain barrier, making it promising for pediatric brain tumor treatment and personalized therapies.

P161

DISTINCTIVE FEATURES OF TUMOR-INFILTRATING γδ T LYMPHOCYTES IN HUMAN GLIOBLASTOMA MULTIFORME

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BACKGROUND-AIM

Glioblastoma Multiforme (GBM) is the most common and deadly primary brain tumor, with a poor clinical outcome which underscores the urgent need for effective therapies. GBM pathogenesis and progression are influenced by the immune microenvironment; hence, a thorough understanding of the immune response within GBM is crucial to better clarify the mechanisms driving tumor development.
This study investigates the role and dynamics of the interaction between tumor cells and γδ T cells in patients with GBM.

METHODS

Both tumor tissue and peripheral blood samples were analyzed trough flow cytometry and, to validate our findings, we data mined an independent cohort of 163 GBM transcriptomes downloaded from the TGCA data set trough GEPIA2.

RESULTS

Flow cytometry and transcriptomic analysis showed that GBM comprised a highly variable rate of tumor-infiltrating lymphocytes (TILs) and 1.3–1.8% γδ T cells, with the majority expressing Vδ2. A similar pattern was detected in the peripheral blood (PB) of GBM patients.
Most Vδ1 and Vδ2 T cells in GBM patients had an effector memory phenotype in both TILs and PB. Conversely, in meningioma patients (used as controls), Vδ1 T cells predominated in TILs, while Vδ2 T cells in PB. Phenotypical analysis showed that Vδ1 T cells had a predominant central memory phenotype, both in TILs and PB, while effector memory Vδ2 T cells predominated in TILs, and central and effector memory Vδ2 T cells were equally distributed in PB.
The transcriptomic analysis showed a correlation between the expression of TRGC1 and TRGC2 (marking Vδ2 and Vδ1 T cells, respectively) and immune checkpoint genes (PDCD1, HAVCR2, TIGIT, and LAG3) in GBM. These data were partially confirmed by flow cytometry analysis: Vδ1 and Vδ2 T cells from TILs of GBM patients expressed high levels of NKG2A and PD-1 and TIM-3, which were slightly expressed or absent in TILs from meningioma patients. Conversely, only NKG2A was expressed by PB Vδ1 and Vδ2 T cells in GBM patients.

CONCLUSIONS

These findings may help to understand the mechanisms of GBM resistance to immunotherapy and identify new therapeutic strategies.

P162

IDENTIFICATION OF MICRORNAS MODULATING ONCOSUPPRESSOR KCASH2 IN COLORECTAL CANCER

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BACKGROUND-AIM

KCASH2 is a negative regulator of the Hedgehog (Hh) signaling pathway, which plays a critical role in both development and tumorigenesis. By promoting the proteasome-dependent degradation of the deacetylase HDAC1, KCASH2 prevents the activation of GLI1, the main transcription factor of the Hh pathway. Given the involvement of aberrant Hh signaling in various cancers, we investigated its potential relevance in colorectal cancer (CRC), one of the most common malignancies, accounting for approximately 10% of all cancer cases and 9% of cancer-related deaths. Although the role of Hh signaling in CRC remains incompletely understood, evidence suggests that GLI1 may contribute to early tumorigenesis. We analyzed the expression levels of KCASH2 and GLI1 in different CRC cell lines. To explore the regulation of KCASH2, we performed an in-silico analysis and identified several microRNAs (miRNAs) with potential binding sites in the 3' untranslated region (3'UTR) of KCASH2. We then confirmed in vitro that some of these miRNAs negatively modulate KCASH2 expression.

METHODS

The human KCASH2 3'UTR and the putative miRNAs involved in its regulation were identified through computational analysis. To characterize the role of these miRNAs in modulating KCASH2 expression, we performed both luciferase, western blot and qPCR assays.

RESULTS

Notably, we observed a decrease in the expression of the KCASH2 mRNA upon overexpression of the selected miRNAs. Consistently, the presence of these miRNAs led to reduction in KCASH2 protein levels, whereas their inhibition resulted in its upregulation. GLI1, the key activator of the Hh pathway, was also monitored following both the overexpression and inhibition of the miRNAs.

CONCLUSIONS

The results shown led to the identification of miR-24 and miR-196b as factors involved in the negative regulation of the KCASH2 tumor suppressor. The discovery of miRNAs as biomarkers may have a significant impact on prevention, early diagnosis, prognosis, and possibly the prediction of therapeutic response. Furthermore, the discovery of new ways to increase KCASH2 levels may lead to novel techniques for suppressing Hh signaling in Hh-dependent cancers, including CRC.

P163

DECODING LONG-TERM RESPONSE TO IMMUNOTHERAPY IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC): INTEGRATIVE ANALYSIS OF PERIPHERAL IMMUNOSCORE AND FUNCTIONAL COMPETENCES OF CIRCULATING T CELLS AND SOLUBLE FACTORS

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BACKGROUND-AIM

Immune Checkpoint Inhibitors (ICIs) is the standard of care for first-line treatment of advanced Non-Small Cell Lung Cancer (NSCLC) lacking targetable oncogenic mutations. Nonetheless, only 20% of patients shows long-term benefits from the treatment and identifying representative biomarkers for monitoring immunotherapy efficacy remains challenging. This study aimed to enable real-time monitoring of immunotherapy response by characterizing peripheral immune components and performing functional analysis of circulating T cells and plasma soluble molecules.

METHODS

Blood immune cell populations of 43 advanced NSCLC patients undergoing ICIs who responded after 1 year of treatment (long responders-LR) or who progressed within the first 3 months (fast progressors-FP) were quantified by cytometer. T cells IFN-γ production was measured after in vitro activation with anti-CD3/CD28/CD2 and IL-2. In vitro activity of immune soluble factors was assessed on NSCLC cell lines growing in 3D.

RESULTS

FP intermediate monocytes exhibited a 50% reduction in HLA-DR expression. In addition, the HLA-DR inhibitor PTX3 resulted significantly increased in FP compared to LR. We also found a 3-fold significant increase of TGF-β in FP's blood. Quantification of IFN-γ production by peripheral T cells showed a significantly higher IFN-γ release in LR compared to FP, suggesting an intrinsically deficient cytotoxic activity of PBMCs in FP and a proficient one in LR.
Furthermore, the impact of the differing IFN-γ production capacities of FP and LR patients was assessed through structural and density analyses of organoids derived from NSCLC cell lines and cultured in the presence of different concentrations of IFN-γ.
Unexpectedly, plasma from FP inhibited 3D growth of ICI-resistant NSCLC cell lines, possibly underlying the presence of autoantibodies and factors potentially capable of interfering with tumor growth.

CONCLUSIONS

Taken together, these results support the possibility of predicting prolonged responses to ICIs by monitoring blood components related to T cell-mediated intrinsic cytotoxicity and monocyte-mediated antigen presentation. The presence of plasma immune components with potential tumor-inhibitory activity highlights the need to further promote immune infiltration within the tumor microenvironment.

P164

ANDROGEN RECEPTOR AND EGFR CROSSTALK IN TRIPLE-NEGATIVE BREAST CANCER: IMPLICATIONS FOR PROGRESSION AND THERAPY RESISTANCE

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer that lacks estrogen, progesterone, and HER2 receptors, which significantly limits targeted treatment options. Because of this, it's crucial to deepen our understanding of the molecular mechanisms behind TNBC to identify new potential therapeutic targets. Among the various signaling pathways involved, the androgen receptor (AR) has emerged as a promising candidate for further investigation. In particular, many studies have demonstrated that understanding the relationship between AR and the epidermal growth factor receptor (EGFR) can provide useful insights into TNBC biology and therapy resistance.

METHODS

We treated TNBC cell lines (HCC1806, BT549) with the synthetic androgen receptor agonist, R1881, and/or EGF, to explore their effects on key cellular processes such as proliferation, migration, epithelial-mesenchymal transition (EMT), and therapy resistance. Cellular responses were assessed using BrdU incorporation assays, wound healing assays, and Western blot analyses.

RESULTS

Contrary to our initial expectations, androgen treatment did not significantly affect proliferation or migration in TNBC cells, while EGF showed opposing effects in the two TNBC cell lines. However, short-term androgen stimulation triggered the activation of several intracellular signaling pathways that might contribute to other aspects of tumor progression. Notably, long-term androgen treatment led to increased expression of EGFR and some EMT markers, suggesting a potential crosstalk between these two signaling pathways.

CONCLUSIONS

Our findings indicate that, in TNBC, AR can control cancer progression and EMT through both rapid and classical pathways. Furthermore, the induction of EGFR expression after prolonged stimulation points to a functional interaction between AR and EGFR. This partnership could be involved in EMT on one side and therapy resistance on the other. Additional research is required to better understand the biological role and therapeutic potential of AR and the possible AR/EGFR crosstalk.

P165

EBNA2 ACTIVATES DNA DAMAGE RESPONSE FOR IMMUNE EVASION

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BACKGROUND-AIM

Epstein-Barr virus (EBV) significantly impacts genomic stability, which is crucial for cellular homeostasis. The DNA damage response (DDR) detects and repairs DNA damage to ensure accurate genetic transmission. The ATM pathway manages double-stranded breaks (DSBs), while the ATR pathway responds to replication stress. EBV manipulates DDR pathways to aid its replication and latency. Viral proteins such as EBNA1, LMP1, and EBNA3C are linked to genomic instability and oncogenesis. Lytic cycle proteins also activate DDR, aiding viral replication. However, the role of EBNA2 in genomic stability remains unclear. Understanding how EBV modulates DDR is vital for revealing mechanisms behind EBV-related cancers and viral immune evasion.

METHODS

To investigate the role of EBNA2 in the modulation of DDR, we utilized diffuse large B-cell lymphoma (DLBCL) U2932 cells and their EBNA2-transfected counterparts. DNA damage and repair processes were assessed using a Spot Counting System to quantify DNA damage, immunoblotting, RT-qPCR, and flow cytometry.

RESULTS

Our data demonstrate that EBNA2 activates DDR by increasing the expression of γ-H2AX, ATM, p-ATM, and its downstream target pCHK2 in DLBCL cells. Inhibition of ATM kinase activity using KU555933 effectively reduced pCHK2 and γ-H2AX induction by EBNA2. Mechanistically, EBNA2 downregulated miR-26a, leading to the upregulation of ATM and pATM. Conversely, LMP1 was found to decrease ATM levels through a strong upregulation of miR-26a. Additionally, we observed that class switch recombination was negatively affected in EBNA2-transfected DLBCL cells.

CONCLUSIONS

Our findings show that EBNA2 activates DDR in DLBCL cells by upregulating ATM through miR-26a suppression. This contrasts with LMP1's effect of reducing ATM via miR-26a upregulation. The negative impact of EBNA2 on class switch recombination further highlights the broader implications in humoral immune evasion by EBV.

P166

HEPATOCYTE DEPLETION OF ERK5 IMPAIRS THE RESPONSE TO LIPOTOXIC OXIDATIVE STRESS RESULTING IN DEFECTIVE INSULIN RECEPTOR SIGNALING

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BACKGROUND-AIM

Insulin resistance is an early event in metabolic dysfunction-associated steatotic liver disease. The MAPK ERK5 has been implicated in the development of hepatic fibrosis and cancer, but its possible role in hepatic insulin sensitivity is unknown.

METHODS

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) of insulin (0.8 U/kg). ERK5 knockdown (KD) in murine hepatocyte cell line (MMH) was performed using lentiviral vectors encoding specific shRNA. Mitochondrial depolarization was assayed using TMRE probe. Quantification of total and mitochondrial ROS was performed using DCFDA and MitoSOX, respectively. OXPHOS metabolism was measured by Seahorse.

RESULTS

ERK5ΔHep mice fed with a HFD exhibited impaired glucose tolerance and reduced insulin sensitivity. In ERK5-KD MMH cells exposed to palmitic acid, to recapitulate in vitro lipotoxicity, insulin-induced activation of Akt was abrogated, and expression of insulin receptor (IR) reduced. Additionally, ERK5-KD induced an increase in mitochondrial ROS, together with activation of JNK, resulting in phosphorylation of IRS1 on inhibitory residues. In ERK5-KD MMH cells and in liver extracts form ERK5ΔHep mice, expression of HMOX1 and NQO1, which mediate the antioxidant response via NRF2, was reduced. Treatment with N-acetyl-cysteine, a free-radical scavenger, prevented downregulation of IR and increased IRS1 phosphorylation. Measurement of mitochondrial membrane potential indicated a strong depolarization in ERK5-silenced cells, together with impaired mitochondrial OXPHOS, associated with up-regulated expression of PGC-1α and TRIB3, a negative regulator of insulin signaling through inhibition of Akt. Reduced expression of IR, and increased expression of PGC-1α and TRIB3 were also observed in liver extracts form ERK5ΔHep mice. In patients with MASLD and insulin resistance, ERK5 expression correlated inversely with LDL levels, and directly with HMOX1 mRNA.

CONCLUSIONS

ERK5 contributes to maintaining hepatocyte insulin sensitivity, via an antioxidant response involving IRS1, PGC-1α, and TRIB3, converging on Akt activation.

P167

COMPARATIVE ANALYSIS OF THE PERFORMANCE OF AUTOMATED DIGITAL CELL MORPHOLOGY ANALYZERS FOR LEUKOCYTE DIFFERENTIATION IN HEMATOLOGIC MALIGNANCIES MINDRAY MC-80 VERSUS WEST MEDICAL VISION HEMA

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BACKGROUND-AIM

The hematology laboratory has enhanced its diagnostic capabilities by using advanced artificial intelligence tools to analyze digital images of peripheral blood cells. The Mindray MC-80 (MC80) has performed excellently in various independent studies.
This study aims to compare the leukocyte differential performance of the MC80 with that of Vision Hema (VH) and the gold standard, manual microscopy.

METHODS

75 patients (M: F 53:47%; median (minmax) age 63 ys (1-90)), with hematological malignancies (ALL=4, CLL=20, AML=20, CML=5, lymphoma=20, infection=6) were analyzed. Their smears were compared using the MC-80, VH, and manual microscopy. According to REF, the agreement between microscopy (reference method, REF), VH, and, MC80, was expressed as the median (IQR) of a given cell population/feature, with REFVH and REF-MC80 differences expressed as bias and 95% limits of agreement.

RESULTS

Concordance was calculated for all complete blood count parameters, but only the following are reported: Neu% [REF: 23.5% (6.5-36.7); REF-VH: 0.09 (-0.35 to 0.54); REFMC80: 0.21 (-1.16 to 1.57)]; Ly% [REF: 45% (12.5-77.8); REF-VH: -2.56 (-6.72 to 1.60); REF-MC80: 23.03 (16.99 to 29.08)]; Mo% [REF: 2.00%(0.50-4.9); REF-VH: -2.15 (-3.57 to -0.73); REF-MC80: -1.47 (-2.42 to -0.51)]; Eo% [REF: 1.0% (0.0-2.0); REF-VH: -0.44 (-0.77 to -0.11); REF-MC80: 0.08 (-0.25 to 0.40)]; Baso % [REF: 0.0% (0.0-0.5); REF-VH: -0.76 (-1.73 to 0.21); REF-MC80: -2.22 (-3.17 to -1.28)]; band cells [REF: 0.5% (0.0-1.5); REF-VH: -0.01 (-0.19 to 0.17); REF-MC80: -1.87 (-2.52 to -1.23)]; myelocytes [REF: 0.00% (0.00-0.5); REF-VH: 0.18 (-0.16 to 0.51); REF-MC80: -4.10 (-5.81 to -2.40)]; metamyelocytes [REF: 0.00% (0.00-0.4); REFVH: 0.33 (0.04 to 0.63); REF-MC80: -0.56 (-0.98 to -0.14)]; blasts, all samples [REF: 0.0% (0.0-34.6); REF-VH: 10.07 (5.17 to 14.97), REF-MC80: -2.05 (-7.06 to 2.96)]; blasts, in acute leukemia [REF: 61.2% (31.5-91.5, 2.0-98.0); REF-VH: 32.55 (21.55 to 43.56), REFMC80: 17.70 (10.18 to 25.23)]; smudge cells in CLL [REF: 64.8% (42.9-100.3); REF-VH: 0.67 (-2.03 to 3.36), REF-MC80: -48.43 (-70.86 to -26.00)].

CONCLUSIONS

The study shows that MC80 has a higher sensitivity in identifying blasts than VH. However, VH shows better agreement with microscopy than MC80.

P168

BREFELDIN A HAMPERS MEDULLOBLASTOMA STEM CELLS FEATURES: UNVEILING A NEW WAY FOR AN OLD MOLECULE

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BACKGROUND-AIM

INTRODUCTION: Medulloblastoma (MB) is a malignant pediatric brain tumor often associated with high resistance to conventional chemotherapy. A subpopulation of tumor cells known as medulloblastoma stem cells (MBSCs) is linked to poor prognosis and therapeutic failure. CD133 (Prominin-1, PROM1) is a transmembrane protein widely recognized as a cancer stem cell marker and correlated with tumor progression. Brefeldin A (BFA), an antibiotic that disrupts Golgi function, induces endoplasmic reticulum (ER) stress responses and has shown antitumor effects in various cancer types. However, its effects on MBSCs remain unexplored. This study aimed to characterize the response of MBSCs to BFA, with a particular focus on its effects on CD133 expression and processing.

METHODS

Group 3 medulloblastoma (G3MB) human cell lines were cultured in B27™ stem-selective medium and treated with 0.3 μM BFA for 24 hours. Following treatment, apoptosis, clonogenicity, and CD133 expression were assessed. CD133 was immunoprecipitated, and the protein bands were analyzed via nanoLC-MS/MS.

RESULTS

BFA treatment led to the overexpression of a CD133 isoform lacking the N-terminal domain. In addition, we observed a downregulation of CD133-mediated intracellular signalling and a reduction in its membrane localization.

CONCLUSIONS

This study provides the first evidence of N-terminal proteolytic processing of CD133, potentially linked to altered intracellular trafficking induced by BFA. These findings offer a proof of principle for developing novel agents with structural and mechanistic similarities to BFA.

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SHARED AND DISTINCT ALTERATIONS IN INNATE LYMPHOID CELLS IN THE GUT OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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BACKGROUND-AIM

Innate lymphoid cells (ILCs) are crucial for maintaining intestinal homeostasis and immune responses. Enriched in mucosal tissues, ILCs maintain barrier integrity and regulate inflammation. Dysregulation of ILCs is linked to inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC). Although ILCs have been studied in IBD, their specific role in disease onset and progression remains unclear. This study aims to analyze the distribution and functional characteristics of ILC1 and ILC3 in IBD patients at disease onset and those with chronic, therapy-resistant inflammation.

METHODS

Mucosal biopsies were collected from three groups: onset IBD patients, therapy-resistant chronic IBD patients, and healthy donors (HD). ILC subsets were analyzed using flow cytometry, confocal microscopy and cytokine production from incubated cell suspensions was evaluated using Luminex assays. Additionally, transcriptomic analysis of online datasets was conducted to assess gene expression changes.

RESULTS

Our findings reveal that both ILC1 and ILC3 (NKp44+ and NKp44−) subsets were increased in IBD patients compared to HD. However, ILC1 frequency was particularly elevated in onset patients, whereas NKp44+ and NKp44− ILC3 were more abundant in the chronic condition.
Functional analysis by intracellular staining demonstrated increased production of IFN-γ and TNF-α by ILC1, as well as IL-17 and IL-22 by ILC3 in both IBD patient groups.
Furthermore, the evaluation of ILC-derived cytokine, chemokines and growth factors exhibited significantly elevated levels of several cytokines, including IL-17, IFN-#, MIP-1# and TNF-#, compared to HD.
Transcriptomic analysis identified the upregulation of inflammatory and ILC-associated genes: RORA, ITGAL, and LEF1, reinforcing the role of ILCs in sustaining inflammation.

CONCLUSIONS

Our findings suggest a dynamic transition from ILC1 to ILC3 predominance during IBD progression, which may contribute to chronic inflammation and treatment resistance. These results highlight the potential of targeting ILC subsets as a novel therapeutic approach for modulating immune responses in IBD.

P170

ANDROGEN RECEPTOR, FILAMIN A, AND CXCR4: PARTNERS IN CRIME IN TNBC

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BACKGROUND-AIM

In triple-negative breast cancer (TNBC), the lack of the classical druggable targets estrogen receptor (ER), progesterone receptor (PR), and type-2 epidermal growth factor receptor (HER-2), makes chemotherapy the primary systemic treatment and a priority for searching for novel therapeutic targets. The androgen receptor (AR) emerges as a promising candidate.

METHODS

Using both in vitro and in vivo models, we investigated how AR and its partners, Filamin A (FlnA) and CXCR4, promote the proliferation and growth of TNBC cells, as well as their impact on the tumor microenvironment (TME). In 2D and 3D models of TNBC-derived MDA-MB-231 and MDA-MB-453 cell lines, together with NIH3T3 stromal cell line, we assessed androgen-induced cell proliferation by measuring 5-Br2'dU incorporation and spheroid growth. By co-immunoprecipitation, immunoblot, and immunofluorescence analyses, we evaluated the mechanisms of action and localization of AR, FlnA, and CXCR4. Transwell assays defined how cancer cells influence stromal cell activity. In both in vitro experiments and MDA-MB-231-BALB/c xenografts, using specific inhibitors designed and synthesized by our and DiSTABiF laboratories, we demonstrated that AR, with FlnA and CXCR4, is responsible for TNBC progression. IHC assays further confirmed that AR action influences cancer cell proliferation and TME composition.

RESULTS

Our results show that androgen treatment triggers cell proliferation in TNBC cells and stromal cells. Furthermore, androgen-treated cancer cells can affect the TME composition. These effects specifically depend on the association between AR and FlnA, which controls both the chemokine receptor CXCR4 and the downstream activation of Ras and PKC. Treatment with the specific AR antagonist Bicalutamide (Casodex, Cx), and two peptides, Rh2025u (mimicking AR sequences responsible for its interaction with FlnA) and R54 (an inhibitor of CXCR4 activity), prevents cancer and stromal cell proliferation and significantly reduces tumor growth in MDA-MB-231-BALB/c xenografts. In summary, our data demonstrate AR's pivotal role in TNBC progression and how this protein can control cancer growth by directly acting on cancer cells and indirectly on the TME.

CONCLUSIONS

These findings highlight AR's significant potential as a molecular target acting on the tumor as a whole. A combined therapeutic strategy to inhibit AR and its "partners in crime" (FlnA and CXCR4) by using our peptides represents a promising approach to develop effective treatments for TNBC.

P171

EXPOSURE TO THE ENDOCRINE DISRUPTORS TRIPHENYL PHOSPHATE AND DIPHENYL PHOSPHATE ALTERS THE DNA BASE EXCISION REPAIR PATHWAY IN NORMAL THYROID CELLS

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BACKGROUND-AIM

Thyroid diseases and carcinogenesis are complex, multifactorial conditions involving thyroid hormone metabolism and oxidative DNA damage. Environmental pollution appears to be the most significant risk factor for the increased incidence of thyroid disease. At present, insufficient data are available on the role of aryl phosphate esters (APEs) metabolite in human thyroid pathophysiology, especially on the ability to interfere with the signals linked to oxidative DNA damage repair. The TPhP metabolite DPhP (diphenylphosphate) was detected in the urine and blood of individuals with thyroid disorders. This study evaluates the molecular effects of exposure to environmental pollutants DPhP and TPhP on a human thyroid follicular epithelium model.

METHODS

We evaluated the effects of aryl phosphate esters pollutans (DPhP and TPhP) on Nthy-ori3-1 cells using the following methods: cell viability by MTS, extracellular production of H_2O_2 by Ros-Glo, ability to internalize pollutant metabolites by HPLC, cytology shape analysis, gene and protein expression of components of the DNA base excision repair system (BER) and EGFR pathway by real-time PCR and Western blot.

RESULTS

Exposure to the metabolite DPhP and the endocrine disruptor TPhP increased mitochondrial activity, hydrogen peroxide production, and cellular stress in proliferating Nthy-ori 3-1 cells. Significant alterations in gene and protein expression of BER components, EGFR, ERbB2 and TPO were observed. The expression of genes involved in BER was modulated by both TPhP and DPhP, exhibiting hormetic, biphasic effects. In particular, DPhP [2μM] induced MUTYH and APE-1/Ref-1 increasing expression, while DPHP [10μM] induced a significant reduction of OGG1 expression. THPH [10μM] increased the expression of ErbB2 and BER proteins, while DPhP [10μM] increased the expression of HER2/ErbB2 and MUTYH in Nthy-ori3-1 cells. The results of this study demonstrate that the DPhP as metabolite of TPhP can induce cellular stress, H2O2 production and alteration of the BER pathway due to bioaccumulation.

CONCLUSIONS

This study is the first to demonstrate that aryl phosphate ester exposure can induce cellular stress and alter the expression of thyroid peroxidase, ErbB2, OGG1, and MUTYH via potential bioaccumulation in the thyroid follicular epithelium. Further studies are needed to better understand the role of pollutant disruptors in dysregulating thyroid function in humans.

P172

TARGETING PATHOLOGICAL ANGIOGENESIS: POSIDONIA OCEANICA EXTRACT MODULATES VEGF SIGNALING AND OXIDATIVE RESPONSES IN HUMAN ENDOTHELIAL COLONY-FORMING CELLS

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BACKGROUND-AIM

Aberrant angiogenesis plays a pivotal role in the pathogenesis of various diseases, including cancer, ocular disorders, and chronic inflammatory conditions. While current anti-angiogenic therapies, primarily targeting the VEGF/VEGFR axis, have shown clinical efficacy, their use is often limited by toxicity, high cost, and the risk of thrombotic and hemorrhagic complications. This has spurred growing interest in natural compounds with multi-targeted, low-toxicity anti-angiogenic potential. Posidonia oceanica extract (POE), a marine-derived phytocomplex rich in antioxidant and anti-inflammatory compounds, has shown promise in modulating endothelial cell functions relevant to angiogenesis. This study investigated the effects of POE on human Endothelial Colony-Forming Cells (ECFCs), which are critical regulators of vascular repair and neovascularization.

METHODS

We investigated the anti-angiogenic, anti-invasive, antioxidant, and anti-inflammatory properties of POE using endothelial colony-forming cells as an in vitro model. POE was obtained through optimized hydroalcoholic extraction and applied at increasing concentrations. ECFC viability, migration, invasion, and tube formation were assessed through standard functional assays. Molecular mechanisms were explored via qPCR and Western blotting to evaluate the expression and phosphorylation of VEGF pathway components and inflammatory/coagulant markers. The reactive oxygen species (ROS) production were quantified using the CellROX fluorescent assay.

RESULTS

POE did not alter ECFC morphology or viability, as confirmed by Trypan Blue and MTT assays. However, functional assays revealed that POE significantly impaired ECFC migration, invasion, and in vitro angiogenesis in a dose-dependent manner. Under VEGF stimulation, POE reduced intracellular ROS accumulation and downregulated key redox-regulating genes. Western blot analysis showed that POE inhibited VEGF-induced phosphorylation of KDR and mTOR, while pAKT and pERK remained elevated, indicating selective disruption of VEGF downstream signaling. Furthermore, POE reduced the expression of pro-inflammatory and pro-coagulant markers and partially reversed TNF-α–induced endothelial activation.

CONCLUSIONS

These findings suggest that POE may counteract oxidative and angiogenic activation in ECFCs, supporting its potential role as a natural, anti-angiogenic agent and lay the groundwork for its further preclinical evaluation in angiogenesis-related disorders.

P173

MECHANOSENSITIVE BMP4 AND THE IL-33/SST2 AXIS IN THE CARDIAC REMODELING OF DIET-INDUCED OBESITY MICE

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BACKGROUND-AIM

Obesity, particularly when induced by chronic consumption of a high-fat diet, is closely associated with adverse cardiac remodeling and increased cardiovascular risk. In this study, we explored the role of Bone Morphogenetic Protein 4 (BMP4), a mechano-sensitive cytokine, and the IL-33/sST2 signaling axis in the hearts of mice with diet-induced obesity (DIO).

METHODS

Male C57BL/6J mice were fed a high-fat diet for 20 weeks to induce obesity and metabolic dysfunction, and were compared with control mice on a standard chow diet. Cardiac tissue was analyzed by qPCR, proteomic assays, and histological evaluation.

RESULTS

Our results revealed a significant upregulation of BMP4 gene in the myocardium of DIO mice. Elevated BMP4 levels were associated with increased expression of hypertrophic markers (ANP, BNP) and pro-fibrotic genes (TGF-β1, collagen I). Interesting, BMP4 expression directly correlates with IL1RL1 gene, which trasduce for sST2, a cardiac maker of maladaptive remodeling.In parallel, we observed an imbalance in the IL-33/sST2 axis: IL-33 expression was markedly decreased, while levels of soluble ST2 (sST2), a decoy receptor that neutralizes IL-33, were significantly elevated. This dysregulation was accompanied by enhanced myocardial fibrosis and infiltration of inflammatory cells, as evidenced by increased gene expression of MCP-1 and IL-6.

CONCLUSIONS

These findings suggest that obesity-induced mechanical and metabolic stress enhances BMP4 signaling and disrupts the IL-33/sST2 cardioprotective pathway, promoting inflammation and structural remodeling of the heart. Understanding these mechanisms may offer novel targets for therapeutic intervention in obesity-related cardiac dysfunction.

P174

UNCOVERING JAGGED1 ACTIVATION BY KRAS AND TAOK3 SIGNALS IN PANCREATIC DUCTAL ADENOCARCINOMA

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BACKGROUND-AIM

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy primarily driven by oncogenic KRAS mutations. Through activation of the MAPK cascade, KRAS signalling intersects with additional pathways, including Notch, contributing to a highly complex oncogenic landscape. Jagged1, a Notch ligand frequently overexpressed in PDAC, has been associated with tumour progression. Our recent findings indicate that KRAS activity promotes the proteolytic processing of Jagged1, generating a transcriptionally active intracellular domain (Jag1-ICD) with oncogenic potential. These data suggest that Jagged1 functions as an integrative node within PDAC signalling networks, warranting further investigation into its molecular role.

METHODS

SiRNA-mediated depletion of KRAS, Notch1, Jagged1 and TAOK3 in PDAC cell lines; pharmacological inhibition using ERK inhibitor (MK-8353) and TAOK3 inhibitor (SBI-581); generation of Jagged1-depleted PDAC lines via CRISPR/Cas9; proliferation assays; mass spectrometry analysis following Jagged1 immunoprecipitation; in vivo xenotransplantation experiments in mice.

RESULTS

We identified a functional KRAS–ERK–Notch1–Jagged1 axis in PDAC: KRAS silencing led to decreased Notch1 activation, reduced Jagged1 expression, and impaired Jag1-ICD processing, supporting a model in which the MAPK cascade sustains Jagged1 expression through Notch1-dependent transcription. Interactome analysis revealed serine/threonine kinase TAOK3, as a novel Jagged1-binding partner. Its silencing or pharmacological inhibition impaired Jag1-ICD release, suggesting a non-canonical post-translational regulatory mechanism. Notably, co-inhibition of ERK and TAOK3 activity impaired PDAC cell viability, indicating that their convergence on Jagged1 represents a functionally relevant vulnerability Consistently, Jagged1 depletion reduced in vitro proliferation and significantly suppressed tumour growth in vivo, reinforcing its oncogenic role.

CONCLUSIONS

Our findings reveal a dual regulatory mechanism of Jagged1 in PDAC, integrating KRAS/Notch1-mediated transcriptional control with TAOK3-driven post-translational processing. Targeting this signalling convergence may offer novel therapeutic opportunities for KRAS-mutant pancreatic cancer.

P175

IFN-γ SHAPES ADAPTIVE TUMOR STATES: IMPLICATIONS FOR RESISTANCE AND THERAPEUTIC TARGETING

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BACKGROUND-AIM

IFN-γ plays a key role in antitumor immunity but can paradoxically promote tumor progression by driving transcriptional and epigenetic remodelling. This dual function remains poorly understood, particularly in the context of resistance to immune checkpoint inhibitors (ICIs). We previously derived two NSCLC primary cell cultures from a single patient: NSCLC-B and NSCLC-H (established before ICI therapy and after progression, respectively). Compared to NSCLC-B, NSCLC-H cells displayed increased features linked to plasticity and invasiveness. This study investigates IFN-γ paradoxical effects on these models exploring alternative therapeutic strategies to overcome ICI resistance.

METHODS

We assessed the effects of IFN-γ, JQ1 (BET inhibitor), 5-azacytidine (5Aza, DNA methylation inhibitor), and NDRG1 silencing under 2D and 3D culture conditions. Functional assays included clonogenicity, sphere formation, and soft-agar growth. NSCLC-B clones were derived from 3D cultures after long-term IFN-γ exposure. scRNA-seq was used to explore transcriptional changes.

RESULTS

Long-term IFN-γ treatment led to NSCLC-H growth inhibition, but increased aggressiveness in NSCLC-B clones, in which scRNA-seq revealed the emergence of molecular heterogeneity under IFN- γ treatment compared to control (10 vs 3 clusters). Shared subpopulations were enriched in IFN-γ–responsive genes associated with tumor plasticity and survival. To overcome ICI-induced tumor plasticity, we tested epigenetic therapies, including JQ1 and 5-azacytidine, on NSCLC-B and NSCLC-H cells. JQ1 showed modest antiproliferative effects on NSCLC-H cells under 2D culture conditions (IC50, 850 nM) but high efficiency in clonogenic and spheroid assays (IC50,17 nM and 40 nM, respectively). 5Aza was more potent in inhibiting NSCLC-B 2D-growth (IC50, 3.39 μM) than NSCLC-H (IC50, 18.78 μM), yet displayed strong activity in NSCLC-H 3D assays (IC50, 0.26 μM).
Of note, inhibition of NDRG1, involved in cell plasticity and lymphocyte activity, also reduced NSCLC-H growth.

CONCLUSIONS

These findings demonstrate how the understanding of the molecular mechanisms driving IFN-γ -induced tumor plastic shifts is essential for designing effective therapeutic strategies to overcome ICI resistance.

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DEVELOPMENT OF A SCORE DERIVED FROM FULL BLOOD COUNT PARAMETERS TO DIFFERENTIATE INDIVIDUALS WITH ACTIVE TUBERCULOSIS FROM THOSE WITH LATENT INFECTION

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BACKGROUND-AIM

In 2023, tuberculosis (TB) caused 1.25 million deaths worldwide, making it the second leading infectious cause of death. Diagnosing TB is challenging, as current tests cannot distinguish between active TB (ATB) and latent TB infection (TBI) leading to potential misdiagnosis or delays in treatment.Research suggests that the monocyte-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, along with absolute counts of various blood cells, could help develop a low-cost and easy-to-use diagnostic tool to distinguish ATB from TBI among IFN-γ release assay (IGRA)-positive subjects without relying on microbiological tests.

METHODS

To develop the TB score, we enrolled 55 with ATB and 57 with TBI, with diagnoses based on WHO criteria and comprehensive clinical assessments. An additional validation cohort of 34 IGRA-positive individuals was included before definitive diagnosis.We evaluated several parameters derived from the complete blood count and additionally assessed specific ratios. For those parameters significantly differing between groups, we constructed a ROC curve and identified a cutoff value optimizing the balance between sensitivity and specificity. Using the cumulative scores assigned to each enrolled subject, we identified a threshold of 8. Individuals scoring greater than or equal to 8 were classified as ATB, while those scoring lower than 8 were considered as TBI.

RESULTS

We have tested our TB on our choort. The score based on eleven blood parameters was able to distinguish TBI and ATB among IGRA-positive subjects with 93% specificity and 71% sensitivity. We validated the TB score in a cohort of 34 hospitalized patients, with a complete blood count and a positive IGRA test as the only available laboratory data. In this cohort, the TB score correctly identified all TBI cases.

CONCLUSIONS

This study highlights the potential of using specific blood cell counts and derived ratios as biomarkers for distinguishing between ATB and TBI and can be useful in supporting the physician's decisions about the therapeutic strategies to adopt for IGRA-positive subjects.

P177

THE COMPLEXITY OF C-MET RECEPTOR ACTIVATION BY DIVERSE LIGANDS AND CO-RECEPTORS

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BACKGROUND-AIM

The often cell-specific pleiotropic effects of HGF/SF, signalling through its tyrosine kinase receptor has baffled scientists since its discovery in the 1980s. By using structural biology and protein engineering and combining it with cell biology, our laboratory has chosen an unusual approach towards the elucidation of this surprisingly complex signalling module that involves heparan sulphate as a co-receptor. Two natural splice variants, NK1 and NK2 have helped our understanding of receptor activation. Both variants have displayed antagonistic and partial agonistic behaviour in vitro. NK1 revealed the important role that heparan sulphate plays on the cell surface. While NK2 also binds heparan, our studies reveal a more complex ability to switch from antagonism to agonism based on the redox state of the environment. We believe this is a novel and unique mechanism to control agonism and link tissue damage to wound healing. More importantly, this mechanism could play a role in the redox-dysregulated tumour microenvironment where it promotes cell survival, proliferation, cell motility, stemness, and drive tumour progression.

METHODS

All proteins, including a mutant form of NK2, were recombinantly expressed in optimised expression systems such as bacterial cells (E. coli BL21), yeast (Pichia pastoris) and mammalian CHO cells. Purified proteins were crystalised in the presence of specific-length purified heparin fragments and used for x-ray diffraction experiments at the ESRF in Grenoble. Biological activity was measured in different fractions from gel filtration chromatography of NK2 and NK2 (C214S) using the sensitive MDCK scatter assay.

RESULTS

For NK2 (C214S), the minimal fragment inducing dimerisation is heparin dp6 and crystals were obtained in complex. Heparin fragments induce dimerisation of monomeric NK1, NK2, and NK2 (C214S), but only NK1 is biologically active. Importantly, activity is observed only in the wild type NK2 fractions underneath the dimer peak and is absent in the heparin dimer of the C214S mutant.

CONCLUSIONS

Smaller natural splice variants have helped us progress towards a mechanistic understanding and allowed the development of several potent agonists and antagonistic molecules for therapeutic application. We continue to focus on the co-receptor heparan sulphate interaction applying structural and biochemical techniques combined with cell biology, to understand better the role of HGF/SF in regeneration and cancer, forming the basis for new therapeutic interventions.

P178

UQCRB INHIBITION OVERCOMES DOCETAXEL RESISTANCE IN PROSTATE CANCER VIA MODULATION OF MITOCHONDRIAL REDOX SIGNALING

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BACKGROUND-AIM

Resistance to Docetaxel remains a significant clinical challenge in the management of advanced prostate cancer. One mechanism implicated in chemoresistance involves hypoxia-driven activation of HIF-1α and downstream survival pathways. UQCRB, a key component of mitochondrial Complex III, regulates mtROS production essential for HIF-1α stabilization. In this study, we assessed whether UQCRB inhibition via Terpestacin could sensitize both Docetaxel-sensitive and -resistant prostate cancer cells, restoring their responsiveness to chemotherapy.

METHODS

Docetaxel-sensitive and Docetaxel-resistant prostate cancer cell lines were exposed to Terpestacin alone or in combination with Docetaxel. Cell viability was determined using MTT assays. Apoptosis was assessed by Annexin V/PI staining and flow cytometry. Changes in cell proliferation were also monitored.

RESULTS

Terpestacin treatment markedly reduced the viability of Docetaxel-resistant cells ($p<0.01$) and significantly enhanced Docetaxel cytotoxicity when used in combination Flow cytometry confirmed increased apoptosis in resistant cells. Notably, Terpestacin alone had minimal effects on proliferation in drug-sensitive cells, underscoring its selectivity.

CONCLUSIONS

UQCRB inhibition by Terpestacin effectively restores chemosensitivity in Docetaxel-resistant prostate cancer cells by disrupting mitochondrial ROS-mediated stabilization of survival signals. These data support UQCRB as a promising therapeutic target for overcoming chemotherapy resistance in prostate cancer and warrant further preclinical evaluation.

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PROSPECTIVE ROLE OF UQCRB INHIBITION IN OVERCOMING HYPOXIA-DRIVEN AGGRESSIVENESS IN THYROID CANCER

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BACKGROUND-AIM

Anaplastic thyroid carcinoma (ATC) is an aggressive malignancy with poor prognosis due to its resistance to therapy, potentially driven by mtROS and HIF-1α stabilization. Here, we present preliminary data on UQCRB inhibition as a novel therapeutic approach for ATC.

METHODS

8505C and BCPAP thyroid carcinoma cells were treated with Terpestacin and Metformin. Soft agar colony formation assays were performed to assess anchorage-independent growth, and cell viability assays measured metabolic activity.

RESULTS

UQCRB inhibition via Terpestacin significantly reduced colony formation and metabolic viability in both ATCC cell lines without evident cytotoxicity, suggesting selective interference with tumorigenic properties. Combination with Metformin further potentiated these effects, implicating cooperative targeting of mitochondrial ROS and metabolic stress pathways. Decreased mtROS levels and increased apoptotic marker expression were observed upon treatment. Ongoing studies aim to elucidate the involvement of HIF-1α and AMPK signaling in mediating these responses.

CONCLUSIONS

These preliminary results support UQCRB inhibition as a potential strategy to mitigate hypoxia-induced aggressiveness in thyroid cancer. Comprehensive molecular characterization will clarify its therapeutic value and guide development of targeted interventions for resistant thyroid malignancies. In vivo validation will be necessary to confirm the translational potential of this therapeutic approach.

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INHIBITION OF HYPOXIA-INDUCED EMT AND STEMNESS IN PROSTATE CANCER CELLS VIA UQCRB TARGETING

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BACKGROUND-AIM

Prostate cancer progression is strongly influenced by hypoxia-induced stabilization of hypoxia-inducible factor 1 alpha (HIF-1α), a transcription factor that promotes epithelial-mesenchymal transition (EMT) and acquisition of cancer stem-like traits. Mitochondrial Complex III-derived reactive oxygen species (mtROS), produced under hypoxic conditions, play a pivotal role in this process by regulating HIF-1α activity. UQCRB, a subunit of Complex III, is critical for mtROS generation. This study evaluated the effects of UQCRB inhibition via Terpestacin on EMT, stemness, and survival markers in DU145 prostate cancer cells exposed to pseudo-hypoxia induced by oncometabolites.

METHODS

DU145 prostate cancer cells were cultured under pseudo-hypoxic conditions and treated with Terpestacin. Prostasphere formation was measured as an indicator of cancer stemness. Western blotting and immunofluorescence analyses were performed to assess the expression of HIF-1α, SOX9, p-AKT, p-STAT3, and NF-κB. Additionally, cell viability assays were conducted to ensure that the observed effects were not due to general cytotoxicity but rather pathway-specific modulation.

RESULTS

Treatment with Terpestacin significantly reduced the number and size of prostaspheres ($p<0.01$), indicating impaired self-renewal ability. Western blotting and immunofluorescence analysis demonstrated a marked decrease in HIF-1α, SOX9, p-AKT, p-STAT3, and NF-κB levels ($p<0.05$). These changes suggest disruption of hypoxia-driven EMT and stem-like phenotype maintenance. No cytotoxic effects were observed in 2D cultures, supporting the specificity of the mechanism.

CONCLUSIONS

UQCRB inhibition by Terpestacin interferes with hypoxia-mediated signaling, EMT induction, and maintenance of cancer stem-like properties in DU145 prostate cancer cells. This highlights UQCRB as a potential molecular target for limiting tumor progression and therapeutic resistance, warranting further validation in vivo.

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PROTECTIVE EFFECT OF SIMVASTATIN ON DOXORUBICIN-INDUCED ACUTE CARDIOTOXICITY: INSIGHTS INTO THE INFLAMMATORY RESPONSE DURING CANCER THERAPY

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BACKGROUND-AIM

Oxidative stress and inflammation are critical contributors to the early onset of Doxorubicin-induced cardiotoxicity. In this study, we explored the potential cardioprotective effects of Simvastatin-well recognized for its antioxidant and anti-inflammatory properties-primary human cardiomyocytes as an in vitro model to evaluate acute Doxorubicin-induced cardiotoxicity.

METHODS

Primary human cardiomyocytes (HCM) cells were pre-treated with Simvastatin (10μM, 4 hrs) and then co-exposed to Doxorubicin (1μM) and Simvastatin for 20 hours. To assess the modulatory role of Simvastatin on Doxorubicin-induced oxidative and inflammatory responses, flow cytometry, ELISA, and quantitative real-time PCR analyses were performed. Data were analyzed by one-way ANOVA followed by Bonferroni post-test.

RESULTS

Simvastatin co-treatment significantly mitigated Doxorubicin-induced oxidative stress, as evidenced by a marked reduction ($p<0.05$) in both cytosolic and mitochondrial ROS levels. Additionally, Simvastatin co-treatment significantly downregulated Doxorubicin-induced Nrf2 gene overexpression ($p<0.005$) and upregulated antioxidant enzymes CAT and SOD2 ($p<0.05$). A significant decrease in nitrite release ($p<0.01$), iNOS gene expression ($p<0.05$), and iNOS protein levels ($p<0.01$) further supported the anti-inflammatory effect of Simvastatin.
Under the same experimental conditions, Simvastatin treatment also attenuated the production of pro-inflammatory cytokines, including TNF-α ($p<0.05$), IL-18 ($p<0.05$), IL-6 ($p<0.005$), and IL-1β ($p<0.05$) as compared to Doxorubicin treatment. Moreover, Simvastatin co-treatment significantly prevented mitochondrial membrane depolarization ($p<0.005$) and apoptosis ($p<0.01$), confirming its protective role against Doxorubicin-induced cardiomyocyte injury.

CONCLUSIONS

These data suggest that Simvastatin could be a valuable additional therapy to reduce Doxorubicin-induced HCM damage, so preventing the development of dilated cardiomyopathy and long-term heart damage, which are the main limitations of anthracycline use.

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THE ROLE OF PATHOPHYSIOLOGY STUDY IN LABORATORY DOCTORS PROFESSIONAL TRAINING

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BACKGROUND-AIM

An acute need for laboratory diagnostics doctors arises in Ukraine during wartime. It is known that pathophysiology is a core subject that helps to understand the mechanisms of genesis, development, and finalizing of any pathological processes and diseases. It is essential to understand the changes in organisms in the course of different diseases, and pathophysiology allows obtaining such skill.
The aim of current work was to estimate the importance of pathophysiology in the professional training of future laboratory doctors.

METHODS

89 students of “Technologies of Medical Diagnostics and Treatment” specialty from known 4 medical universities were involved in research. A sociological survey was carried out with the use of Google Forms questionnaires distributed via social networks.

RESULTS

78% of respondents were aware of the importance of pathophysiology; the other 22% reported that this subject was secondary in the study. Additionally, 43% explained their choice by the impossibility to predict any changes in the organism without understanding the pathophysiological process; the others had difficulties with that opinion. 68% gave the practical example when pathophysiology was helpful (like cases of blood values alteration during iron deficiency anemia were described to the patient); the rest gave no examples. Most negative answers were motivated by disappointment in their chosen profession (35%); disappointment in medical university (28%); changes in study priorities (21%); low academic motivation (14%); and conflict with teacher (2%).

CONCLUSIONS

There is certain incomprehension among students regarding the importance of pathophysiology in professional training. Also, there are factors connected with changes in educational path that can affect proper understanding of pathophysiology role.

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WAR IMPACT ON RESPIRATORY FAILURE OCCURRENCE AMONG STUDENTS

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BACKGROUND-AIM

Cardiovascular, respiratory, and oncological diseases have increased their prevalence among population of Ukraine in recent wartime years. One of the leading positions in breathing system disorder structure belongs to respiratory failure. A lot of research of respiratory failure pathogenicity still cannot completely describe its development during war.
The aim of present work was the determination of war impact on prevalence of respiratory failure symptoms in students.

METHODS

Sociological survey was carried out among 90 students of KNMU (80 males, 10 females). Most respondents lived in frontline proximity towns of Ukraine and were affected by war factors. Google-forms questionnaire was composed and distributed via social networks.

RESULTS

The analysis of war factors impact on respiratory failure was showed, that 89% respondents underwent chronic stress; main reason of stress was respondents' alarm for themselves, in other cases – for relatives in frontline. 77% mentioned the worsening of their health in past year. Main symptoms among respondents were breathlessness and constrained feelings in chest during physical activity (44,4%); dry cough (33,3%); breathlessness even at rest or low physical activity (12,2%), and complicated breathing-out (10,1%). 44,4% of respondents mentioned that at least 5 times a day they have to take cover in badly ventilated shelters. Rating of factors that inflict mentioned symptoms according to respondents was the following one: wartime stress (70%), smoking (17%), hypodynamia (7%), alcohol consumption (5%), and, finally, bad ration (1%).

CONCLUSIONS

Adverse impact of wartime can be presumed through increased occurrence of respiratory failure signs among youths.

P184

EXPLORING THE ROLE OF GLYCATED ALBUMIN AS A MARKER OF GLYCATION STRESS IN CHRONIC KIDNEY DISEASE PATIENTS WITH AND WITHOUT DIABETES MELLITUS

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BACKGROUND-AIM

Chronic kidney disease (CKD) is a major clinical condition associated with an elevated risk of cardiovascular events. This increased risk arises from a multifactorial etiology, involving both traditional (e.g., diabetes mellitus) and non-traditional mechanisms, such as inflammation, accumulation of uremic toxins and oxidative stress-induced dysfunctions. Glycemia and oxidative stress plays a key role in promoting the early glycation of circulating proteins, including albumin, and the synthesis of advanced glycation and products (AGEs). In CKD, AGEs accumulation also occurs due to the reduced kidney filtration. Although both glycated albumin (GA) and AGEs are products of glycations, due to the different mechanisms regulating their synthesis, they could not be related each other. The meaning of their use as biomarkers of glycation stress needs therefore to be fuly explored.

METHODS

We evaluated cross-sectionally 122 CKD patients (stages 3a-5). Demographic, clinical and biochemical data were collected. We quantified GA, AGEs, inflammatory markers, and the soluble receptor for AGEs (sRAGE) on serum samples. Data were compared between diabetes and non-diabetes patients and correlated with the other parameters.

RESULTS

The main result is that GA exhibited no correlation with AGEs or systemic inflammation or sRAGE. Notably, this lack of correlation persisted even after stratification according to the presence of diabetes. GA reflected glycemic status and correlated with glycated hemoglobin in diabetes patients only.

CONCLUSIONS

In conclusion, in CKD patients, GA seems to be more useful as an early marker of glycemic control than a marker of glycation stress, which is the result of multiple factors, including reduced filtration and inflammation.

P185

AN INTEGRATED SIGNALING NETWORK CONTROLS GLIOBLASTOMA GROWTH

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BACKGROUND-AIM

Human gliomas, including glioblastoma (GBM), are characterized by high percentage of mortality and morbidity. Although major advances have been recently made to identify relevant players in the regulation of glioma growth and development, so far infiltrating gliomas, including GBM, are incurable.

METHODS

We used an integrated approach of gene/protein targeting strategies aimed to selectively interfere with key elements in the signaling networks activated by G protein-coupled receptors and evaluate the biological consequences of this genetic and pharmacological manipulation on GBM growth, both in cultured cells and preclinical models.

RESULTS

We have discovered that activation of GPCR-cAMP-PKA cascade profoundly modifies the stability of many proteins involved in cell growth, development and metabolism. The levels and the activity of several of these proteins are significantly altered in GBM cells, suggesting that their action is important for GBM development, progression and drug resistance. At mechanistic level, we found that the ubiquitin pathway regulated by cAMP converges on the RING ligase praja2, which regulates the stability and activity of a variety of downstream effector proteins implicated in the metabolic rewiring, autophagy, protein translation and senescence of GBM cells. We have assembled a praja2 molecular network operating in GBM cells that underlies critical aspects of tumor biology.

CONCLUSIONS

We provided proof-of-principle that interfering with the praja2-ubiquitin system in cultured cells, organoids and xenograft models of GBM is a valid therapeutic approach to restrain tumor growth.

P186

CBX2 AS A POTENTIAL TARGET FOR THERAPEUTIC INTERVENTION IN CRC

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BACKGROUND-AIM

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. This study investigates the involvement of CBX2 and its domains in CRC progression.

METHODS

We adopt a reverse genetic approach to explore the role of CBX2 in CRC cell lines employing shRNA targeting CBX2. Next, to address the underlying molecular mechanisms of CBX2-suppressed phenotype, transcriptome-wide analysis has been performed. Additionally, to gain insight on the molecular role of CBX2 protein domains, CBX2 mutants were generated in which deletion and/or point mutations were inserted in each domain. Afterwards, to explore their impact on CBX2 function and chromatin regulation ChIP-seq and ATAC-seq experiments will be performed.

RESULTS

Our investigation reveals that CBX2 is overexpressed in CRC primary samples compared to the normal counterpart. Interestingly, we highlighted that CBX2 silencing negatively impacts CRC cell growth triggering cell death. Additionally, CBX2 knockdown inhibits the migration capabilities of CRC cells, effectively inhibiting their invasiveness. Intriguingly, RNA-seq showed a downregulation of gene sets associated with crucial pathways for CRC survival (such as EF2 targets and TGFβ pathway) and, on the contrary, enrichment of genes related to apoptosis induction.

CONCLUSIONS

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.

P187

MICRO- AND NANOPLASTICS INDUCE DYSREGULATION IN HUMAN MONOCYTES: INSIGHTS INTO AUTOPHAGY AND INFLAMMATION

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BACKGROUND-AIM

The presence of micro- and nanoplastics (MNPs) has become a growing environmental and health concern due to their widespread presence and potential biological impact. Chronic exposure to MNPs in humans occurs via ingestion and inhalation, and they are putatively linked to inflammation and immune dysregulation. However, the molecular and cellular mechanisms underlying these effects remain to be fully elucidated as long as a direct causative effect with any pathology. The aim of our research is to define the effect of MNPs on the immune system.

METHODS

In this study, we evaluated the impact of both polystyrene (PS) MNPs and UV-aged PS-MNPs of different sizes (0.5, 1, 5 µm) on freshly isolated primary human monocytes. Overall, the focus was to examine their effects on key immunological and cellular pathways, including inflammation, endocytosis, mitochondrial function, and the autophagy-lysosomal system.

RESULTS

Functional assays revealed a size- and dose-dependent reduction in cell viability and an increase in apoptosis following MNPs exposure. Our results demonstrated that monocytes internalize MNPs via endocytosis, resulting in their accumulation within lysosomes. This internalization process led to the formation of a multilamellar morphology, accompanied by a substantial accumulation of autophagosome. Our results highlighted upregulation of key markers involved in autophagy, lysosomal function, cellular activation, inflammation and altered expression of genes involved in mitochondrial fission and recycling. Furthermore, we observed a concomitant downregulation of antigen presentation and inflammasome activity. Notably, UV-aged MNPs triggered more intense inflammatory and cytotoxic responses than pristine particles.

CONCLUSIONS

In conclusion, our findings provide novel insights into the immunological and intracellular impact of MNPs on human monocytes. MNPs impair immune cell function by disrupting mitochondrial and lysosomal integrity, inducing autophagy, and activating inflammatory pathways. Notably, UV-aged MNPs exert more pronounced cytotoxic and pro-inflammatory effects compared to pristine particles, suggesting that environmental degradation enhances their immunotoxic potential. These findings highlight the potential health risks associated with MNPs and stress the importance of further research into their long-term effects on human health. Current limitations of the field will be highlighted and discussed as well.

P188

INTRACELLULAR P2 RECEPTORS AS MODULATORS OF LIPID METABOLIC REPROGRAMMING IN CLEAR CELL RENAL CELL CARCINOMA

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BACKGROUND-AIM

Clear cell renal cell carcinoma (ccRCC) is characterized by profound alterations in lipid metabolism, including lipid droplet accumulation, dysregulated fatty acid oxidation, and aberrant cholesterol handling, which sustain tumor growth, immune evasion, and therapy resistance. The study aims to investigate how mitochondrial and lysosomal P2Rs regulate lipid metabolic reprogramming in ccRCC and to evaluate their contribution to tumor progression and resistance mechanisms, using advanced proteomic and lipidomic approaches.

METHODS

ccRCC tissue samples and cell models were analyzed to quantify P2R expression and their impact on metabolic pathways. Proteomic profiling was performed using high-resolution mass spectrometry to assess the expression of P2Rs and key metabolic regulators, including AMPK, mTOR, and SREBPs. Lipidomic analysis was conducted via mass spectrometry-based lipid quantification to characterize alterations in lipid droplet content, fatty acid composition, and cholesterol distribution. Mitochondrial function was assessed through β-oxidation assays and ROS measurements, while lysosomal activity and lipophagy were evaluated using fluorescence microscopy and biochemical assays.

RESULTS

Proteomic analysis revealed upregulation of mitochondrial and lysosomal P2Rs in ccRCC samples compared to adjacent normal tissue. Lipidomic data demonstrated increased lipid droplet accumulation, impaired fatty acid oxidation, and altered cholesterol trafficking, consistent with a lipid-rich tumor phenotype. Functional assays confirmed that P2R signalling modulates mitochondrial β-oxidation efficiency, ROS production, and lysosomal lipophagy, contributing to metabolic flexibility, anabolic support, and resistance to nutrient stress. These findings suggest that targeting P2R-mediated pathways may disrupt lipid-driven tumor progression and offer new therapeutic avenues in ccRCC.

CONCLUSIONS

Our data support the role of mitochondrial and lysosomal P2 receptors as key regulators of lipid metabolic reprogramming in clear cell renal cell carcinoma. By influencing fatty acid oxidation, lipid storage, and cholesterol homeostasis, P2Rs promote the metabolic plasticity required for tumor progression and therapeutic resistance. Targeting these intracellular purinergic pathways may represent a promising strategy to impair lipid-dependent tumor growth and improve treatment efficacy in ccRCC.

P189

INFLAMMATORY AND FIBRINOLYTIC MARKERS IN CYSTIC FIBROSIS SPUTUM - EFFECT OF SAMPLE PROCESSING

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BACKGROUND-AIM

Cystic fibrosis (CF) is a genetic disease whose hallmark is a chronic inflammation of the airways causing tissue damage and increased levels of alarmins in the respiratory secretions. The coagulation system activation is known to be linked to inflammation and involved in CF. However, no information is available about the fibrinolytic system and how fibrinolysis is involved in airway injury. On the road to understanding this issue, we determined whether dithiothreitol (DTT), a reducing mucolytic agent regularly used to homogenise sputum, affects the detection of two key markers, HMGB1, an alarmin, and D-dimer, associated with fibrin breakdown and airway inflammation, in the sputum from such patients.

METHODS

Sputum samples have been collected from 13 individuals with CF (either homozygous or compound heterozygous for the F508del mutation). Sputum samples were subjected to two different treatment protocols. In the first protocol, after mechanical removal of saliva, the sample was treated with DTT and then centrifuged in order to collect the supernatant (hereinafter referred to as SED). In the second protocol, after removal of saliva, the sample was centrifuged and the supernatant (hereinafter referred to as SE). The pellet obtained was treated with DTT and then centrifuged in order to collect the supernatant (hereinafter referred to as SPE). ELISA assays were performed on all samples.

RESULTS

HMGB1 and D-dimer levels were lower, though not significantly, in supernatants from sputum centrifuged before DTT treatment (SE) compared to those processed after DTT (SPE and SED). Differences between SE vs. SPE and SE vs. SED approached statistical significance, particularly for HMGB1 and D-dimer in SE vs. SPE. However, no significant difference was observed between SPE and SED samples for either marker.

CONCLUSIONS

CONCLUSIONS
Our results suggest that sample processing steps may influence the detectability of inflammatory biomarkers in CF sputum. It appears that DTT treatment may promote the progressive release of the inflammatory mediators from the mucus matrix; it is likely that, in the absence of the mucolytic action, each mediator may be partially bound to the proteins of the mucus matrix. Samples treated with DTT will be obtained from other CF patients and all samples will be analysed for other fibrinolytic factors.
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P190

β-GAL+CD56+CD3-NK CELLS AND β-GAL SERUM LEVELS WERE FOUND ELEVATED IN A GROUP OF MULTIPLE SCLEROSIS (MS) PATIENTS.

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BACKGROUND-AIM

Multiple Sclerosis (MS) is a chronic immune-mediate neuroinflammatory disorder affecting the Central Nervous System (CNS). Its clinical presentation and disease course are highly variable ranging from clinically isolated syndrome (CIS), relapsing-remitting (RR) to primary progressive (PP). Since studies suggest that autoimmunity can be associated with premature immunosenescence (IS) we decided to investigate the possible physiopathological mechanisms of MS progression and therapy resistance involving IS to identify biomarkers of MS activity with particular attention to BPIFB4 and β-Galactosidase (β-Gal), well-known senescence-related protein.

METHODS

Patients (N=20) were enrolled in the study at disease onset (T0) and every 6 months for two years they underwent follow-up, together with Healthy Controls (HC) (N=10). For each patient were collected blood, serum, plasma, PBMCs and, where needed, CSF. Immunophenotype was performed on PBMCs. Serum and CSF have been used to assess β-Gal, Plasma for BPIFB4.

RESULTS

Fluorescence activated cell sorting (FACS) analysis from 16 RR patients and 4 CIS patients showed that the NK population consists of 19,73±8,27% of β-Gal+CD56+CD3-NK in MS patients compared to 10,7±0,7% of senescent β-Gal+CD56+CD3-NK in 10 HC (t test=0,02). The exhaustion markers (CD39, PD-1, Tim3) didn't show differences among the two groups. Moreover, no differences were found in the T CD3+ cell compartment. Surprisingly β-Gal serum levels were significantly higher in RR patients (20013±7650pg/mL) compared to both CIS (12104±4840pg/mL) and HC (9270±6329pg/mL) both at T0 and T1. On the contrary, β-Gal was barely detectable at CSF level. Accordingly, plasma BPIFB4 levels in both RR (677,4±241,4pg/mL) and CIS (409,6±238pg/mL) at T0 have been demonstrated to be higher if compared to HC (362,8±145,8pg/mL) (p value=0,0008).

CONCLUSIONS

These preliminary data confirm the involvement of IS NK in MS pathology. In the near future, the correlation with time of disease evolution and its severity score might add translational value to the senescence-related immune signature for MS diagnosis and follow up.

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DEVELOPMENT OF A 3RS-BASED SYNOVIUM-ON-CHIP PLATFORM FOR MODELLING RHEUMATOID ARTHRITIS AND PREDICTING TREATMENT RESPONSE

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BACKGROUND-AIM

Rheumatoid arthritis (RA) is a chronic autoimmune disease primarily affecting the synovial joints, with a global prevalence of ~0.5–1%. Despite many available therapeutic options, more than 40% of patients still face long-term disability and significant social costs due to the absence of predictive tools for treatment response. To address this gap, we developed a patient-specific synovium-on-chip (SoC) platform using RA cells from minimally invasive biopsies. This 3Rs-compliant model enables personalized clinical trials-on-chip to predict drug efficacy.

METHODS

A 3D microfluidic platform was designed to host autologous, leukocyte-infiltrated synovial microtissues embedded in 5% Gelatin Methacrylate (GelMA). The chip integrates a pressure-driven actuator to mimic joint loading. Synovial and blood-derived RA cells were encapsulated and bioprinted, then cultured under inflammatory conditions (IL-6, IL-1β at 10 ng/mL, and TNF-α at 20 ng/mL) to mimic the in vivo inflammatory microenvironment in RA. To demonstrate the potential of the platform, a pilot clinical trial-on-chip assessed their response to methotrexate and anti-TNF-α, widely used therapeutic agents in RA treatment. We evaluated cell viability, proliferation, phenotype, lubricin production, cytokine and extracellular vesicle (EV) secretion, and single-cell transcriptomics and proteomics.

RESULTS

The SoC supported the formation of viable 3D synovial microtissues replicating key RA features. Fibroblast-like synoviocytes (FLS) differentiated into lining and sublining phenotypes with different morphology and functionality depending on spatial distribution. Mechanical stress upregulated lubricin secretion, while inflammation suppressed it. The platform allowed in vitro patient-specific drug testing, revealing donor-dependent cytokine modulation. Multiplex cytokine analysis (more than 50 cytokines) and Principal Component Analysis (PCA) highlighted inter-individual variability in response to methotrexate and anti-TNF-α, supporting predictive capacity.

CONCLUSIONS

This autologous human SoC replicates essential aspects of RA pathophysiology and drug response. It enables rapid, personalized in vitro testing, reduces reliance on animal models, and supports the identification of patient pathotypes. Beyond therapy prediction, the SoC offers a versatile platform for studying RA heterogeneity and disease mechanisms.

P192

THE ONCOLYTIC ADENOVIRUS DL922-947 INDUCES IMMUNOGENIC CELL DEATH IN TRIPLE NEGATIVE BREAST CANCER CELLS

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, characterized by poor prognosis and limited therapeutic options due to the lack of estrogen and progesterone receptor and HER2 expression. The development of novel and more effective treatment strategies is therefore imperative. Oncolytic viral therapy has emerged as a promising alternative approach in cancer treatment. dl922-947 is a selectively replicating oncolytic adenovirus, engineered with a 24 base pair deletion in the E1A-Conserved Region 2 (CR2). This modification restricts viral replication to cells with a defective pRb/G1-S cell cycle checkpoint, an abnormality common in the majority of human cancers. In this study, we aimed to evaluate the anticancer efficacy and cell death mechanisms induced by dl922-947 in TNBC cell lines models.

METHODS

TNBC cell lines (MDA-MB-231, DU-4475, and MDA-MB-468) were treated for 72h with dl922-947. Cell viability was assessed using MTT assay. Apoptosis/necrosis were evaluated by staining the cells with Annexin V/PI, followed by flow cytometry (FC) analysis. ICD markers such as calreticulin cell surface exposure and HMGB1 levels were analyzed by FC, whereas a luminescence-based bioassay assessed ATP content. Additionally, we evaluated dl922-947 effects on IL-6 secretion by ELISA assay. The phagocytic activity of THP-1 human leukemia monocytic cell line was evaluated by FC after 24-hour incubation with conditioned medium (CM, 1:2 dilution) collected from MDA-MB-231 cells treated with dl922-947 for 6 days.

RESULTS

We compared the sensitivity of different cancer cell lines to dl922-947-induced cytotoxicity and we observed higher efficacy in MDA-MB-231 and MDA-MB-468 cells that we used for further experiments. We have demostated that dl922-947 infection induces apoptosis and modulates the main hallmarks of ICD, inducing calreticulin surface exposure, increased HMGB1 levels and ATP release in both cell lines. We observed modulation of IL-6 secretion in MDA-MB-231 cells and the CM collected from these cells in the presence of the virus increased phagocytosis of THP-1 cells.

CONCLUSIONS

dl922-947 induces anticancer effects in TNBC cells and can drive the activation of an anti-tumor immune response. Thus, we suggest that oncolytic viral therapy might represent a promising therapeutic strategy for TNBC treatment. Further studies are required to investigate the TNBC cell lines different response to dl922-947 and to expand our cells panel to include more TNBC cell lines.

P193

VERIFICATION OF A CE IVD NGS ASSAY FOR ESR1/PIK3CA DETECTION IN CFDNA FROM METASTATIC BREAST CANCER PATIENTS

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BACKGROUND-AIM

Breast cancer (BC) remains the most frequently diagnosed malignancy in women worldwide. Liquid biopsy has emerged as an attractive, minimally invasive alternative. Blood-based assays analyse surrogate material such as circulating cell-free DNA (cfDNA) and circulating tumour DNA (ctDNA). Several next-generation sequencing (NGS) platforms are available for ctDNA profiling; among them the PlasmaSeqSensei#Breast#Cancer#IVD assay detects mutations in multiple targeted genes, including#ESR1 and PIK3CA. The clinical utility of ctDNA-detected ESR1 mutations for anticipating endocrine resistance has been established and such testing is now essential for selecting patients eligible for SERDs (e.g. elacestrant). The aims of this study were to verify the performance of the CE-IVD kit and to evaluate its application in a real-world cohort of BC patients.

METHODS

A total of 12 positive control samples were analysed. Peripheral blood was collected in Streck Cell-Free DNA BCT tubes. cfDNA was isolated using the QIAamp#Circulating#Nucleic#Acid Kit (Qiagen) and libraries were prepared with the PlasmaSeqSensei#Breast#Cancer#IVD kit. Sequencing was performed on an Illumina platform; variant calling was carried out with the accompanying#IVD software, reporting both mutant allele fraction (MAF) and absolute mutant molecule counts (MM). Subsequently, the assay was applied to cfDNA from 57 patients with metastatic BC.

RESULTS

The PlasmaSeqSensei#Breast#Cancer#IVD assay performed as expected on all positive control samples, confirming its reliability for detecting known ESR1 and PIK3CA mutations. Variants not included in prior panels, such as TP53 and KRAS, were also identified. In the clinical cohort (n=57), ESR1 mutations were detected in 23 patients, including recurrent alterations such as Y537S (n=7), D538G (n=10), and combined Y537S+D538G (n=3), along with other rare variants (e.g. L536K, E380Q). PIK3CA mutations were observed in 21 patients, with E545K being the most frequent (n=12), followed by H1047R (n=4), and additional alterations including N345K, H1047L and multimutational profiles. In total, 35 patients harbored at least one clinically relevant mutation.

CONCLUSIONS

The PlasmaSeqSensei#Breast#Cancer#IVD assay showed strong robustness, reproducibility, and clinical utility. In real-world use, it reliably detected expected and actionable mutations, supporting its routine use in cfDNA profiling for metastatic BC.

P194

MAML1 DRIVES NOTCH AND HEDGEHOG ONCOGENIC PATHWAYS BY INHIBITING ITCH ACTIVITY IN TRIPLE-NEGATIVE BREAST CANCER

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is an aggressive and heterogeneous breast cancer subtype with poor patient outcomes. Its heterogeneity arises from multiple dysregulated pathways, including Notch and Hedgehog, which contribute to tumor initiation, progression, and drug resistance. Identifying common molecular regulators of TNBC aggressiveness is crucial for developing effective therapeutic strategies. Here, we demonstrate that the transcriptional coactivator MAML1 drives TNBC aggressiveness by regulating Notch1 and Gli1 stability through the E3 ubiquitin ligase Itch, functioning as an Itch-negative regulator.

METHODS

Immunoprecipitation and ubiquitination assays in both in vitro and ex vivo cell lines; analysis of Itch post-translational modification; siRNA-mediated depletion of Maml1 in breast cancer cell lines; proliferation assays; PLA; in vivo orthotopic transplantation and tail-vein injection experiments in mice.

RESULTS

MAML1 interacts with Itch via its PPQY motif and promotes K63-linked self-ubiquitylation of Itch, deregulating its expression/activity. Using a Maml1-deficient mouse model, we reveal an inverse correlation between MAML1 and Itch levels, where the loss of MAML1 stabilizes Itch and suppresses Notch1 and Gli1 activity. Conversely, MAML1 upregulation enhances Notch1 and Gli1 expression, driving accelerated TNBC tumor growth and faster multiorgan metastasis in vivo. Accordingly, we show that MAML1 is overexpressed in a cohort of TNBC patients, and the combined overexpression of MAML1/Notch1 and MAML1/Gli1 correlates with poor clinical outcomes by in silico analysis.

CONCLUSIONS

Our findings establish a dual role for MAML1 as a transcriptional coactivator and a post-translational regulator of Itch, thereby amplifying Notch and Hedgehog oncogenic signaling. This study uncovers MAML1 as a key driver of TNBC progression and a potential therapeutic target for fighting TNBC aggressiveness and heterogeneity.

P195

TARGETING MIR-24 TO RESTORE TUMOR IMMUNOGENICITY IN EBV-ASSOCIATED B-CELL LYMPHOMAS

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BACKGROUND-AIM

Epstein-Barr virus (EBV) contributes to the development of immunoblastic lymphoma and is involved in the pathogenesis of diffuse large B-cell lymphoma (DLBCL). The EBV-encoded nuclear antigen 2 (EBNA2) promotes immune evasion by dysregulating microRNA expression, reducing the immune co-stimulatory molecule ICOSL through the induction of miR-24, and increasing the immune checkpoint PD-L1 by suppressing miR-34a. This dual mechanism supports immune escape and cellular proliferation. Developing therapies that target these miRNAs could be an effective approach to counteract EBV-driven immunosuppression, especially in tumors with high levels of miR-24.
Our aim is to restore tumor immunogenicity by reducing miR-24 levels in a B-cell lymphoma model, thereby increasing ICOSL expression.

METHODS

We used two types of chemically modified oligonucleotide compounds: fourteen modified locked nucleic acids (LNAs) targeting miR-24 and a peptide nucleic acid (PNA) targeting miR-24. Non-targeting oligonucleotides served as controls. Different concentrations of these compounds were tested for their ability to derepress ICOSL in the U2932 DLBCL and EBNA2-transfected cell lines. Reverse transcriptase quantitative PCR (RT-qPCR) and flow cytometry (FACS) were used to evaluate changes in miR-24 and ICOSL expression. As a functional assay Luciferase reporters of the 3' UTR of ICOSL were used to study the direct suppression of ICOSL by miR-24 compounds. MTT assays were performed to assess any toxic effects of the oligo controls and miR-24 inhibition in the cell lines.

RESULTS

Anti-miR-24 compounds were successfully delivered to the cells at both concentrations (50 nM and 100 nM for LNA and 1μM for PNA). We observed no toxicity from the oligo controls. Increased luciferase activity of the 3' UTR ICOSL reporter confirmed that miR-24 inhibitors directly repressed ICOSL via its 3'UTR. The compounds at the chosen concentrations were not toxic to the cell lines. We ultimately selected the most effective concentration of the miR-24 compound to restore ICOSL expression in EBNA2-positive lines.

CONCLUSIONS

Our findings indicate that inhibiting miR-24 restores ICOSL expression, potentially improving immune recognition in EBV-associated B-cell lymphomas. These results support the development of miRNA-based therapies to enhance tumor immunogenicity and advocate for the use of precision medicine strategies in EBV-driven cancers.

P196

TWO-STEP IN VITRO MEMORY TEST TO ASSESS SARS-COV-2-SPECIFIC IMMUNE RESPONSE

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BACKGROUND-AIM

A booster shot is an additional vaccine dose that prolongs immunity. To determine whether humoral/cellular immune responses against SARS-CoV-2 are sufficient to prevent re-infection and make a booster unnecessary, we developed a two-step in vitro "MEMORY TEST."

METHODS

Step1: Neutralizing activity (NA) to SARS-CoV-2 was assessed in plasma samples (n°=100) from SARS-CoV-2 infected/vaccinated (SI) or uninfected/unvaccinated (SU) subjects. PBMCs of 37 SI and 10 SU subjects were in vitro stimulated with inactivated SARS-CoV-2 for 5 days to assess B and T cell memory induction. Step2: A549-ACE2 cells were SARS-CoV-2 infected (MOI:0.001). After 1-hour supernatants or PBMCs derived from step1 were added to A549-ACE2 infected cells to test if induced SARS-CoV-2 specific humoral/cellular immunity are sufficiently protective to control viral replication over time (48, 72h).

RESULTS

According to their NA, SI subjects were divided in high responders (SIH; NA≥320) or low responders (SIL; NA≤80). After step1, CD4+, CD8+ T cells and B cells from SU did not significantly change their phenotypic or functional profile. Conversely, in SI re-exposure to SARS-CoV-2 stimulated both T and B cell maturation. Specifically, we observed: 1) a significant reduction in naïve B cells ($p<0.0001$), an increase in the percentage of plasmablast CD20+/CD27+/CD38+($p<0.05$),

Notably, viral replication in A549-ACE2 cells was not affected in the presence of either supernatants or PBMCs from SU derived from step1. Contrarywise, viral infection/replication was significantly reduced in the presence of both PBMCs (48 and 72h: $p<0.0001$) and supernatants (48 and 72h: $p<0.0001$) from SI. Such protective immunity was independent from initial NA as SIH and SIL displayed the same trend in both step 1 and 2.

CONCLUSIONS

These findings suggest that humoral and cellular memory triggered by SARS-CoV-2 re-exposure can control viral replication, regardless of NA. Our two-step in vitro MEMORY TEST reliably predicts immune responses to re-exposure, offering a valuable tool to assess the need for booster doses, particularly in immunocompromised individuals, such as PLWH.

P197

IN VITRO MODULATION OF IMMUNE CHECKPOINTS BY IMMUNOSUPPRESSIVE DRUGS USED IN TRANSPLANT REJECTION PREVENTION

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BACKGROUND-AIM

Organ transplant rejection is primarily driven by adaptive immune responses targeting mismatched HLA molecules. Immunosuppressive therapies are key in preventing rejection, but recent findings highlight the role of immune checkpoint (IC) molecules in modulating alloimmune responses. This raises the question of whether different immunosuppressive drugs influence IC expression. We investigated the effect of commonly used immunosuppressants on the expression of key IC molecules in vitro.

METHODS

Peripheral blood mononuclear cells (PBMCs) from eight healthy donors were co-cultured with A549 lung epithelial cells for 24 hours to model alloimmune interactions. PBMCs were either pre-treated with azathioprine (10 µM, 1 µM, 100 nM), basiliximab (50 µg/mL, 10 µg/mL, 1 µg/mL), or tacrolimus (100 ng/mL, 50 ng/mL, 10 ng/mL), or the drugs were added directly to the co-culture. T lymphocytes expressing TIM3, GAL9, PD-1 and CTLA-4 (%), as well as A549 expressing GAL9 were assessed by flow cytometry.

RESULTS

Azathioprine significantly reduced the frequency of CTLA-4-expressing T lymphocytes at the highest concentration, regardless of whether PBMCs were pre-treated ($p < 0.001$) or the drug was added directly to the co-colture ($p < 0.0001$). Similarly, pre-treatment with tacrolimus (100 ng/mL) lowered CTLA-4 expression ($p < 0.05$). Conversely, co-culture with immunosuppressants induced a compound-specific increase in IC-expressing T lymphocytes: 1) azathioprine - 10µM: PD-1 $p<0.05$, GAL9 $p<0.05$; 2) basiliximab - 50µg/ml: PD1 $p<0.01$, GAL9 $p<0.05$, 10µg/ml: PD-1 $p<0.01$, GAL9 $P<0.01$, 1µg/ml: PD-1 $p<0.05$, GAL9 $p<0.001$; 3) tacrolimus - 50ng/ml GAL9 $p<0.05$, 10ng/ml: PD-1 $p<0.05$. Additionally, co-culture with basiliximab (50µg/mL) significantly increased the frequency of GAL9-expressing A549 cells after 24 hours ($p < 0.05$).

CONCLUSIONS

Immune checkpoints are emerging as critical regulators of graft tolerance. Our findings demonstrate that immunosuppressive drugs used in lung transplantation differentially modulate IC expression in vitro. These drug-specific effects may have important implications for fine-tuning immunosuppressive regimens to promote graft acceptance and warrant further investigation in translational and in vivo studies.

P198

GENOMIC PROFILING OF COLLOID CARCINOMA OF THE PANCREAS

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BACKGROUND-AIM

Colloid carcinoma (CC) of the pancreas is a rare and distinct subtype of pancreatic cancer (PDAC), representing 3% of all pancreatic tumors. Unlike the more common PDAC, CC often arises from intraductal papillary mucinous neoplasms (IPMNs) and is distinguished by the abundant extracellular mucin produced by tumor cells. While CC shares similarities with other pancreatic tumors in terms of symptoms, diagnosis, and treatment, it has a significantly better prognosis than PDAC. At the molecular level, CCs are associated with frequent mutations in GNAS, with MSI cases typically being KRAS wild-type. However, most molecular studies focus primarily on PDAC, with relatively few cases of CC. The reasons behind its improved prognosis remain unclear. The aim of this study is to characterize IPMN-associated CC

METHODS

We used DNA-targeted sequencing (174 genes) and immunohistochemistry (IHC) analyses.

RESULTS

This study analyzed 46 patients, including 27 with IPMN-associated CC (1 with intraductal oncocytic papillary neoplasm, IOPN; 26 with IPMN) and 19 with CC alone. Histological grading was performed based on immunophenotypic classification to characterize these tumors. The IHC analyses confirmed that IPMNs are mostly high-grade intestinal lesions, while the IOPN exhibited only high-grade dysplasia. Among the invasive tumors, 31 out of 46 (67%) were classified as muconodular and low-grade tumors, whereas 15 out of 46 (33%) displayed an infiltrative glandular component and high-grade tumors. DNA sequencing revealed that the most common genetic variations affected KRAS (48%), GNAS (46%), TP53 (35%), ATM (22%), RNF43 (17%), and CDKN2A/SMAD4 (15%). Interestingly, 4 out of 9 ATM mutations were germline, as confirmed by Sanger sequencing. In addition to these germline mutations, one patient had a CHEK2 mutation, and another carried two different germline mutations affecting NF1 and ATM. Two patients of 46 showed microsatellite instability (MSI) with high tumor mutational burden (TMB). Lastly, it was found patients harboring amplification in CCND1 (1/46), CCND2 (4/46), CCND3 (1/46), CCNE1 (1/46), and IDH2 (1/46), which may be sensitive to targeted therapies (CDK4/6 inhibitors, CDK2 inhibitor or IDH2 Inhibitors).

CONCLUSIONS

Overall, this study, with the largest cohort of patients with CC, emphasizes the value of genomic profiling to identify patients with 1) potentially druggable mutations, gene amplifications, or MSI, and 2) germline mutations sensitive to platinum-based therapies and/or PARP inhibitors.

P199

EXTRACELLULAR VESICLES CHARACTERIZATION IN PATIENTS WITH HEART FAILURE WITH PRESERVED OR REDUCED EJECTION FRACTION

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BACKGROUND-AIM

Heart failure (HF) is a clinical syndrome involving structural and/or functional cardiac abnormalities, classified as reduced (HFrEF) or preserved (HFpEF) based on the ejection fraction percentage of the left ventricle. As extracellular vesicles (EVs) reflect onset and severity of cardiac diseases, they attract interest as potential liquid biopsies. Aim of the present project was to characterize EVs in HFrEF and HFpEF, investigating their potential as biomarkers and discriminating factors in HFrEF and HFpEF with a particular focus on the underlying molecular mechanisms.

METHODS

The study included 39 HF patients (13 HFpEF and 26 HFrEF) and 31 volunteers (CTR). EVs were isolated from plasma by size-exclusion chromatography and ultracentrifugation, then characterized using nanoparticles tracking analysis, transmission electron microscopy (TEM), Western blot (WB) and flow cytometry (FACS). ELISA assays were used to measure circulating proteins. Functional assays were performed to assess the impact of patient-derived EVs on a cellular model of monocyte.

RESULTS

Diagnosis of HF relied on echocardiographic (e.g. E/e' ratio) and biochemical parameters (e.g. NT-proBNP). Patients with HF were characterized by raised circulating markers of fibrosis (Gal-3, VEGF-C, YKL-40). When HFrEF and HFpEF groups were considered, Gal-3 and YKL-40 were significantly raised only in HFrEF compared to CTR. Isolation of EV was confirmed by FACS and WB analyses (CD63, CD9, CD81, Alix and β1 integrin), while integrity by TEM. EV size was increased in HF (nm: 202 vs 181). Among different subpopulations of EVs, those from monocytes ($CD14^+$), neutrophils ($C66b^+$), endothelial cells ($Cd202b^+$) and activated endothelial cells ($CD62E^+$) were significantly reduced in HF. Treatment of monocytes (THP-1) with EVs derived from HF patients led to an increased expression of proinflammatory cytokines (i.e. IL-1α, IL-1ß, IL-6 and IL-8), when compared to cells treated with EVs isolated from CTR subjects. This change was mostly driven by EVs derived from HFpEF patients.

CONCLUSIONS

EVs derived from HF patients exhibit a distinct profile that reflects the hemodynamic characteristics of the condition and possess proinflammatory properties.

P200

DISSECTING THE ROLE OF PIN1-NOTCH3 AXIS: A NEW "TUNABLE" MEDIATOR OF PLATINUM RESISTANCE IN HIGH-GRADE SEROUS OVARIAN CANCER

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BACKGROUND-AIM

Resistance to platinum-based drugs represents a major obstacle for the management of high-grade serous ovarian cancer (HGSOC) patients. Indeed, the selective pressure of platinum-based (PT) chemotherapy often leads to the outgrowth of platinum-resistant subclones. The underlying adaptive networks should be fully investigated to provide advances toward more streamlined and personalized care. In this scenario, Notch3 is altered in a wide panel of OC primary samples, and it sustains PT-resistance, thereby supporting that Notch3 targeting may overcome PT-resistance. We demonstrated that Pin1 positively regulates Notch3 in T-cell acute leukemia and we wondered whether this relationship occurs in HGSOC. Overall, the main aim is to investigate whether and how Pin1-Notch3 axis is involved in PT-resistance in HGSOC.

METHODS

We conducted a comprehensive analysis of Pin1/Notch3 relationship from HGSOC cell lines to primary cells and tumours, integrating multiple Pin1 genetic targeting under chemotherapy pressure, differential proteomic approaches, molecular docking data and dynamics simulations, thus identifying a functional circuit evaluated in vitro and in vivo models.

RESULTS

Here, we demonstrated that carboplatin treatment of HGSOC cells promoted the activation of the Pin1/Notch3 axis, resulting in the upregulation of β-catenin and platinum resistance. Neoadjuvant chemotherapy treatment of HGSOC-bearing patients is correlated with increased Pin1/Notch3 (and β-catenin) expression and PT-resistance acquisition. Conversely, genetic targeting of Pin1 combined with carboplatin treatment sensitizes resistant cells to platinum-based therapy, both in vitro and in vivo, strongly reducing their Notch3-mediated metastatic potential in preclinical murine models. Mechanistically, Pin1-Notch3 binding favours protection of Notch3 from its GSK3β-mediated degradation, resulting in increased Notch3 expression and β-catenin interaction.

CONCLUSIONS

Collectively, our findings identify the functional Pin1/Notch3 (β-catenin) axis as an escape strategy from chemotherapy-induced cell death, thus suggesting a novel predictive role of the Pin1/Notch3 (β-catenin) axis in the platinum response, which could be useful for implementing frontline treatments for HGSOC patients before recurrence.

P201

EXTRACELLULAR VESICLES AS PROGNOSTIC MOLECULAR BIOMARKERS OF CHEMOTHERAPY-INDUCED CARDIOTOXICITY

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BACKGROUND-AIM

With advancements in cancer therapy improving survival rates, there is growing awareness of the short- and long-term complications of these treatments, including cardiovascular toxicities that impact morbidity and mortality. Although cardiotoxicity is evaluated using echocardiographic left ventricular global longitudinal strain and circulating cardiovascular biomarkers, it remains unclear whether different biomarker criteria yield varying incidence and risk estimates. Aim is to explore whether extracellular vesicles (EVs) represent a blueprint approach in identifying biomarkers for detecting early onset of chemotherapy-induced cardiotoxicity. We focused on female breast cancer, the most common cancer globally, with a 5-year survival rate of up to 88%.

METHODS

We gathered data from 11 women (age range: 30-69 years) diagnosed with breast cancer who underwent anthracycline chemotherapy. Cardiological evaluations were conducted before (T0) and three months after chemotherapy (T1), assessing cardiac biochemical variables and imaging evaluation. EVs were isolated from plasma by size-exclusion chromatography and ultracentrifugation, then characterized using nanoparticles tracking analysis, transmission electron microscopy, and flow cytometry (FACS).

RESULTS

Regarding biochemical parameters, only cardiac troponin showed a significant increase [T0: 5.03 ng/L (4.25;7.51) vs T1 9.39 ng/L (7.39; 15.6); p= 0.0031], while for echocardiographic evaluation, only E/e' was significantly raised [T0: 6.51 (4,89;6,9) vs T1: 7,58 (6,73;8,44); p= 0.0452]. Regarding EVs, no significant differences were observed in size and quantity. When examining the cellular origin, only EVs released from cardiomyocytes (CD172+), neutrophiles (CD66b +) and platelets (CD41a+) showed differential expression. These EV changes induced by anthracycline chemotherapy were independent from change in plasma neutrophils or platelets count. Notably, the decrement in the EVs derived from cardiomyocytes at follow-up was positively correlated with the rise in cardiac troponin (r= 0.4467; Spearman correlation).

CONCLUSIONS

Characterizing EVs at the subcellular level may serve as a liquid biopsy tool capable of detecting early stages of anthracycline-induced cardiotoxicity.

P202

THE IMPACT OF EBV-ENCODED BILF1, A POORLY KNOWN G-PROTEIN COUPLED RECEPTOR GENE, ON THE PATHOGENESIS OF BURKITT LYMPHOMA

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BACKGROUND-AIM

Burkitt lymphoma (BL), which arises from germinal centre B cells (GCB), is an aggressive non Hodgkin B-cell lymphoma. The hallmark of nearly all BL tumours is the chromosomal translocation between the MYC gene and one of the immunoglobulins (Ig) heavy or light chain loci. According to the World Health Organization (WHO), BL can be classified into three forms which differ in geographic distribution, clinical presentation, and Epstein–Barr virus (EBV) association: endemic (eBL), sporadic (sBL) and HIV-associated BL. The association with EBV is highly variable, with more than 90% of the endemic cases and near 30% of HIV-associated tumours linked to EBV. The sporadic form is rarely associated with EBV, with only 10-15% cases diagnosed as EBV-positive. The majority of BL tumours express a latency type I, characterized by the expression of only EBNA1, EBV-encoded BART miRNAs and the non-coding RNA-pol III non-translated RNAs termed EBV-encoded small RNAs (EBER)-1 and EBER-2EBER RNAs.
However, other latent and lytic transcripts such BILF1 have been reported in a subset of BL cases. The function of these virus transcripts remains largely unkown.
Here we have identified a novel role for the EBV-encoded BILF1, a constitutively active viral G-protein coupled receptor with transforming ability in in vitro (NIH3T3 cells) and in vivo (nude mice), in the BL pathogenesis.

METHODS

High throughput Q-PCR assay and RNA in situ hybridisation revealed that BILF1 is expressed by most tumour cells of a subset of eBL.
RNAseq of BILF1-transfected cells showed that BILF1 induces a transcriptional programme that recapitulates the aberrant transcriptional programme characteristic of primary eBL.

RESULTS

In contrast to our current knowledge, BILF1 is expressed in the latently infected tumour cells of a subset of eBL. Moreover, when expressed in primary human GC B cells (the progenitors of eBL), BILF1 induces a transcriptional programme that recapitulates the aberrant transcriptional programme characteristic of primary eBL, including the up-regulation of known MYC and P13-K target genes.

CONCLUSIONS

Our data indicate that BILF1 induces an oncogenic transcriptional programme that could be important for the pathogenesis of a subset of eBL.

P203

MODULATION OF REDOX BALANCE AND INDUCTION OF CELL DEATH BY EVOO PHASE II METABOLITES IN INTESTINAL CANCER CELL LINES

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BACKGROUND-AIM

The link between diet and cancer has been proven by numerous studies, and a healthy diet including daily consumption of extra virgin olive oil (EVOO) is known to participate in the prevention of the onset of cancer due to its content in phenolic compounds. The main goal of this research work was to highlight possible anticancer effects of EVOO phenolic hydroxytyrosol (HT) and tyrosol (TYR), together with their main phase II metabolites.

METHODS

Tyr glucuronide (Tyr glu), Tyr sulfate (Tyr sulf), HT glucuronide (HT glu) and HT sulfate (HT sulf) were tested (0.5 - 10 μM) on Caco-2 and HT-29 colorectal cancer cell lines. Cell viability was evaluated via the MTT assay, while inhibition of cell proliferation and induction of apoptosis, as well as effect of the treatment on cell cycle, were assessed through flow cytometric analyses. 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.

RESULTS

Caco-2 cells showed moderate sensitivity to HT and HT sulf from 2.5 μM, while no significant effects emerged for Tyr and other metabolites which were effective in HT-29. The ability to induce cell death by apoptosis, was assessed at appropriate amounts, based on the MTT assay results. Caco-2 cells showed changes caused by Tyr glu of late apoptotic and necrotic cells, an increase in the G1 phase and a reduction in the G2/M phase of cells treated with all the compounds; in HT-29 cells significative effects were observed only after HT-sulf incubation. This compounds also induced a 2-fold increase of GSH/GSSG ratio in Caco-2, while no effects were observed in HT-29.

CONCLUSIONS

This study highlighted the anticancer effects of the main phase II metabolites of HT and Tyr, able to induce apoptosis, dysregulate cell cycle phases and modulate oxidative stress in Caco-2 and HT-29 cells. The results suggest that these compounds contribute to the beneficial effect exerted by EVOO in the prevention of chronic diseases and cancer.

P204

EVALUATION OF NEUROTROPHIC EFFICACY IN IN VITRO MODELS OF GLAUCOMA: INSIGHTS INTO OXIDATIVE STRESS, INFLAMMATION, AND CANDIDATE MIRNA BIOMARKERS

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BACKGROUND-AIM

NGF biological mechanism in preventing retinal neurodegeneration is not completely understood. Availability of suitable retinal in vitro models is crucial for NGF response analysis. Age and elevated intraocular pressure (IOP) are the two primary risk factors for glaucoma, the leading cause of irreversible blindness. However, mechanisms at the base of glaucoma development and strategies possibly able to revert this condition are still unknown. Response to NGF was evaluated in two in vitro retinal models of glaucoma by analysis of oxidative stress/inflammation factors and miRNAs' expression levels to identify new candidate biomarkers/therapeutic targets.

METHODS

RPE and rMC-1 cells were cultured using an original device, under basal and high pressure (HP) condition, the latter mimicking glaucoma. Cells were treated with NGF for 96h, then oxidative stress and inflammation markers were analyzed by immunoblotting. Starting from miRNA profiling results, expression levels of the most interesting microRNAs were analyzed by qRT-PCR.

RESULTS

HP condition induced NRF2 and HO-1 increase in RPE and rMC-1. After NGF treatment, significant reduction of NRF2 and HO-1 and slight CAT and SOD-1 decrease was observed. Similarly, IL-6 and IL-1β levels increased in HP condition to decrease after NGF treatment. MiR-29c-3p, miR-146a-5p, miR-34a-5p, whose levels increased in HP condition to decrease after NGF, were analyzed. Bioinformatics analysis highlighted several pathways known to be involved in glaucoma pathogenesis, oxidative stress, inflammation and apoptosis, as well as interesting target genes (e.g. SIRT1, NRF2, HO-1, DJ-1, SOD-1, CAT, IL-6, IL-1β, TNF-α).With this regard, the interaction between miR-34a-5p and SIRT1, a gene involved in inflammation and oxidative stress, was validated. As expected, results showed SIRT1 decrease in HP condition which was reverted after NGF treatment.

CONCLUSIONS

In the models here described, NGF induces a decrease in oxidative stress and inflammatory responses due to HP condition. Enrichment of miR-29c-3p, miR-146a-5p, miR-34a-5p target genes and pathways strongly indicates their involvement in HP responses, suggesting a putative role as potential glaucoma biomarkers or therapeutic targets, and opening for further studies to validate these findings.

P205

IPSC REPROGRAMMING IDENTIFIES NOVEL CANDIDATE DRIVERS OF PRELEUKEMIC CLONAL EVOLUTION

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BACKGROUND-AIM

Myeloid neoplasms are clonal disorders driven by somatic mutations in hematopoietic stem cells (HSCs). Driver mutations confer competitive advantage to HSCs leading to clonal expansion, termed clonal hematopoiesis (CH). However, nearly 50% of CH cases lacks an annotated driver, implying that the number of drivers might be larger than catalogued to date. CH infrequently progresses to leukemia and preleukemic clones are rare in leukemic samples. Thus, processes underlying transformation from CH to malignancy remain unclear.

METHODS

We used induced pluripotent stem cell (iPSC) reprogramming to isolate clonal intermediates from 18 patients with myeloid neoplasms and track genomic and functional evolution of preleukemic clones. To measure mutational dynamics, we performed whole exome sequencing of primary samples, preleukemic iPSCs, and wild-type iPSCs. To functionally test candidate drivers, we knocked-down selected genes in primary human HSCs.

RESULTS

We generated 42 iPSC lines spanning all stages of preleukemic evolution marked by 1, 2, or 3 drivers and isogenic wild-type clones from the same patient. Preleukemic mutations increased linearly with clonal progression. While in a neutral process the ratio of amino acid-changing to neutral mutations remains constant, we found that 2- and 3-drivers clones harbored a significantly higher proportion of amino acid-changing mutations compared to 1-driver clones, suggesting selection for genes not annotated as drivers. To identify unrecognized drivers, we selected genes expressed in the bone marrow and with pathogenic variants in our dataset. Of these, 19 genes were mutated at a 0.03 – 1% frequency in myeloid neoplasms, with NFE2, NSD1, TET1 and IRF1 being the most frequent. Among them, the histone methyltransferase NSD1 is a fusion oncogene in chromosomal translocations in pediatric leukemia and inactivating mutations are known drivers in squamous cell carcinoma. We found that depletion of NSD1 significantly affected hematopoiesis, promoting expansion of phenotypic HSCs.

CONCLUSIONS

These findings suggest that mutations in non-annotated driver genes can promote HSC expansion and nominate NSD1 as a new driver. We propose that somatic mutations in a large number of genes previously unrecognized as drivers contribute to preleukemic clonal evolution.

P206

TUNING LAT1 EXPRESSION: A NEW ROUTE TO POTENTIATE BNCT IN OSTEOSARCOMA

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BACKGROUND-AIM

Boron neutron capture therapy (BNCT) is an innovative cancer treatment based on the selective accumulation of boron-10 in tumor cells, followed by irradiation with thermal neutrons. This triggers a nuclear reaction that produces high linear energy transfer (LET) particles, causing localized cytotoxic effects while sparing healthy tissues. BNCT efficacy relies on efficient intracellular uptake of boron-containing compounds, mainly mediated by the L-type amino acid transporter 1 (LAT1, encoded by SLC7A5), which transports large neutral amino acids and borate analogues. LAT1 is frequently overexpressed in aggressive tumors, including osteosarcoma, where it supports metabolism, redox homeostasis, and DNA repair, contributing to therapy resistance. Targeting LAT1 could enhance osteosarcoma sensitivity to BNCT.

METHODS

To modulate LAT1 expression in osteosarcoma cells, two complementary strategies were applied. First, SLC7A5 was silenced using siRNAs to evaluate the biological consequences of LAT1 suppression. Second, small-molecule epigenetic modulators (HDAC and DNMT inhibitors) were used to induce LAT1 expression. Following LAT1 modulation, transcriptomic profiling by RNA sequencing was performed to identify gene expression changes. Functional enrichment and transcription factor activity analyses were conducted to explore affected pathways. In addition, boron uptake was assessed by mass spectrometry in LAT1-silenced and control cells.

RESULTS

LAT1 silencing affected pathways related to oxidative stress, DNA repair, and cell cycle control. Transcription factor analysis revealed reduced activity of RELA, NFKB1, JUN, and FOXO1, key regulators of genotoxic stress responses. LAT1 depletion impaired adaptive mechanisms needed for tumor survival and significantly reduced boron uptake (BPA), confirming LAT1's essential role in BNCT efficacy. Conversely, pharmacological induction of LAT1 increased boron uptake and enhanced cytotoxicity under simulated BNCT conditions. The dual approach allowed precise modulation of LAT1, shedding light on its role in both tumor biology and treatment response.

CONCLUSIONS

LAT1 plays a critical role in regulating DNA damage response and radiation sensitivity in osteosarcoma. Its modulation, via silencing or pharmacological induction, represents a promising strategy to enhance BNCT effectiveness. The identification of epigenetic mechanisms controlling LAT1 expression opens new therapeutic perspectives, supporting further evaluation in preclinical in vivo models.

P207

MIMICKING THE "BRCANESS" CONDITION IN OVARIAN CANCER

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BACKGROUND-AIM

Ovarian cancer (OC) is the fifth leading cause of death among women, especially the High grade Serous Ovarian Carcinoma (HGSOC) subtype, frequently resulting in resistant to chemotherapy and relapse. 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 to mimic this condition is the Pin1 isomerase inhibition as Pin1 binds and colocalizes with BRCA1 in breast cancer cells, increasing its stability under genotoxic stress. Thus, its depletion reduces HR process, sensitizing BRCA1-proficient cells to PARPis. Our main aim is to examine the Pin1-BRCA1 relationship in Ovarian Cancer context.

METHODS

BRCA1 proficient established HGSOC cell lines were used. We performed: i. 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, Mass Spectrometry-based proteomics, Immunofluorescence analysis, lentiviral inducible Pin1 genetic targeting, transient transfections, pharmacological Pin1 inhibition, cell viability assay) and iii. in vivo experiments (xenografts transplant in NSG mice)

RESULTS

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, both in vitro and in vivo.

CONCLUSIONS

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, thus extending the overall survival of patients regardless their BRCA-mutational status.

P208

TREGS DERIVED EXTRACELLULAR VESICLES ARE ASSOCIATED WITH IMMUNOSUPPRESSIVE ACTIVITY AND PREVENT GVHD IN MURINE MODELS

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BACKGROUND-AIM

In HLA-haploidentical transplantation, T regulatory cells (Tregs) co-infused with conventional T cells (Tcons), protect against graft-versus-host disease (GvHD) while maintaining the graft versus leukemia effect. Extracellular vesicles (EVs) emerged as a promising alternative for cell-based therapies. In this study we isolated Tregs-derived EVs for the first time and characterized them for their in vitro and in vivo activities.

METHODS

Immunoselected Tregs (CD4+CD25+, FoxP3+, CD127+) were activated with T Cell TransAct and expanded with Rapamycin and IL-2. Tregs-derived EVs were isolated by fluorescence activated cell sorting (patent n. EP19164567A). EVs were characterized by nanoparticle tracking analysis (NTA), atomic force microscopy (AFM), TEM and western blotting. A proteomic approach and bioinformatic analysis were applied for EV protein cargo study. Tregs-derived EV inhibition potential was assessed by suppression assay on autologous Tcons. A GMP-compliant separation method by anti-CD3 immunomagnetic beads was developed, achieving a 70% EV recovery rate. NSG mice were irradiated with 2.5 Gy and were infused weekly with human Tcons and with Tregs-derived EVs or human Tregs (control). Before sacrifice, mice were scored for GvHD.

RESULTS

Treg-derived EVs had a globular shape, ranged in size from 50-150 nm and expressed EV-specific markers (CD63, CD81 and Flotillin-1), while cytochrome C, a negative protein marker of EVs, was exclusively detectable in Treg cell lysate and absent from EV fractions. Proteomic analysis of Treg EVs identified proteins connected in functional networks that are related to "Immune system" and "Immune response". A 24-hour suppression assay showed a potent immunosuppressive effect, with inhibition rates of 97–98% observed when 3–6×10# EVs were applied per 1×10# Tcons. Inhibition effects of Treg EVs were comparable to those induced by the related parental cells ($p<0.05$). Furthermore, in humanized murine models, Treg-derived EVs effectively prevented GvHD, while maintaining the beneficial graft-versus-leukemia response mediated by Tcons.

CONCLUSIONS

These findings support the potential of Treg-derived EVs as a novel immunotherapeutic agent with GvHD prevention.
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P209

KCASH2 EXPRESSION LEVEL IN COLORECTAL CANCER DATASETS AND IDENTIFICATION OF NOVEL REGULATING CANDIDATE MIRNAS

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BACKGROUND-AIM

Sonic-Hedgehog (Hh) pathway plays a crucial role in embryonic development, tissue differentiation and staminality. Altered Hh signaling is involved in medulloblastoma, basal cell carcinoma, rhabdomyosarcoma, and growing evidence links deregulation to colorectal cancer (CRC). Gli, final Hh effectors, are the most studied factors. Histone deacetylase HDAC1 was shown to modulate Hh signaling, deacetylating Gli1/2 and enhancing transcriptional activity. KCASH protein family binds HDAC1 deacetylase, inducing its ubiquitination and degradation, and KCASHs overexpression was demonstrated to suppress Hh pathway and tumor cell proliferation. This study focuses on investigating KCASH2 involvement in CRC and on its microRNA-mediated regulation.

METHODS

A publicly available database (GENT2) was interrogated to evaluate potential associations between KCASH2 expression level and clinicopathological/molecular characteristics. TaqMan-based microRNA profiling was performed in 5 CRC cell lines showing different KCASH2, Gli1 and HDAC1 protein levels. The strategy was based on the comparison between cells with high (LS174T, Caco2) and low (SW48, HCT116, HT29) KCASH2 protein expression. An additional comparison was made between SW48 and Caco2 cells, showing opposite expression patterns of KCASH2 and GLI1/HDAC1. Data was analyzed by Expression Suite software and differentially expressed miRNA targeting KCASH2 were in silico identified.

RESULTS

Datasets' analysis evidenced a subset of CRC patients showing KCASH2 downregulation, mostly linked to KRAS/BRAF wild-type status, high chromosomal instability (CIN), and low CpG island methylation phenotype (CIMP). Integrative analysis identified 18 miRNAs targeting KCASH2. MiR-24-3p, miR-196b-5p, miR-27-3p emerged as the best candidate, being already described in CRC and reported in different databases, also experimentally supported.

CONCLUSIONS

CRC datasets' analysis highlighted a subset of patients (KRAS/BRAF-WT, high-CIN, low-CIMP) with KCASH2 downregulation. MiRNA profiling of CRC cells, showing different KCASH2 expression levels, evidenced 3 miRNAs (miR-24-3p, miR-196b-5p, miR-27-3p) putatively involved in KCASH2 downregulation, opening for further analyses to validate their functional role in CRC.

P210

ROLE OF OXIDATIVE STRESS AND NEUROINFLAMMATION IN A GLAUCOMA MOUSE MODEL. IN VIVO EVIDENCES FOR A NEW THERAPEUTIC APPROACH.

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BACKGROUND-AIM

Glaucoma is a chronic optic neuropathy and the leading cause of irreversible blindness, affecting 80 million people globally. Glaucoma is characterised by progressive degeneration of retinal ganglion cells (RGCs), their optic nerve axons, and synaptic loss at dendrites and axon terminals. Currently, lowering IOP is the main therapeutic approach for slowing disease progression in patients with glaucoma.
While reducing intraocular pressure (IOP) - one of the major risk factors for glaucoma - is the gold standard of glaucoma treatment, in many cases glaucoma continues to progress despite IOP reduction. Therefore, additional therapeutic strategies are still greatly needed for vision preservation or restoration in glaucoma patients, in particular novel approaches that are effective in protecting RGCs from degeneration (neuroprotection), boosting their functions (neuroenhancement) or counteracting oxidative stress (antioxidant).
The aim of this study is to determine the potential therapeutic efficacy of neurotrophic factor (NTF) to counteract glaucoma.

METHODS

An in vivo study was carried out by using DBA/2J mice, widely used as mouse model of ocular hypertension. NTF was injected intravitreally and Electroretinogram (ERG), Optometry tests and molecoular analysis (Rt-qPCR, western blotting and IHC) has been performed

RESULTS

No distress signs were observed during the study as well as no death suggesting the absence of systemic toxicity following NTF treatment. Although NTF does not demonstrated efficacy in ameliorating IOP, significant increase of the visual acuity and scotopic a-wave amplitude were reported in the glaucomatous animals treated with NTF. Histological evaluation showed a significant reduction of retina thickness and RGC count in mice treated with vehicle compared to NTF. Molecular analysis showed a reduction of marker of gliosis as well as a strong decrease of proinflammatory cytokines and apoptosis after NTF injection.

CONCLUSIONS

Our results demonstrate that NTF ameliorates visual acuity loss, preserves retinal thickness, sustain RGC survival, and reduces neuroinflammation, offering a multifaceted approach to combat glaucomatous damage.

P211

NON-CANONICAL ACTIVATION OF HEDGEHOG SIGNALING BY FUSOBACTERIUM NUCLEATUM CONTRIBUTES TO STEMNESS IN CRC THROUGH AUTOPHAGY PROMOTION

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BACKGROUND-AIM

Colorectal cancer (CRC) recurrence, metastasis, and resistance to therapy is sustained by a subpopulation of colorectal cancer stem cells (CR-CSCs). Current therapies fail to eradicate these cells, underlining the need for targeted strategies. Fusobacterium nucleatum (FN), a Gram-negative anaerobe bacterium belonging to the oral microbiota, is enriched in CRC patients and associated with chemoresistance and poor prognosis. Interestingly, FN has been shown to bind CR-CSCs in vitro via the adhesion molecule CEACAM1, activating oncogenic pathways such as ERK1/2. However, the downstream signaling events connecting FN infection to increased stemness and therapeutic resistance remain unclear.

METHODS

To assess FN's effect on CRC cell stemness we used organoid formation, evaluated stemness marker expression, and analyzed HH pathway activation in both differentiated and patient-derived CR-CSCs. We also used genetic and pharmacological inhibition approaches to investigate the molecular mechanism.

RESULTS

Using an organoid formation assay, we demonstrated that Fusobacterium nucleatum (FN) infection increases organoid size and upregulates stemness marker expression. Co-culture of colorectal cancer stem cells (CR-CSCs) with FN led to a dose-dependent upregulation of the Hedgehog-associated transcription factor GLI1 and its downstream targets. Notably, genetic or pharmacological inhibition of ERK1/2 not only prevented FN-induced organoid growth but also suppressed GLI1 upregulation, supporting the involvement of an ERK1/2–GLI1 axis in the regulation of cancer cell stemness.

Given that autophagy is a key modulator of stem cell function, we next assessed whether FN could induce autophagic flux. We found that FN enhances autophagic activity in CR-CSCs, but only in the presence of GLI1. This effect was not altered by Vismodegib, an inhibitor of the canonical Hedgehog pathway, indicating that FN promotes autophagy through a non-canonical GLI1 activation mechanism.

CONCLUSIONS

FN promotes CRC stemness by activating GLI1 via ERK1/2 and induces autophagy to support tumor cell survival. Due the importance of CR stem cells in metastasis and tumor recurrence, these findings lay the groundwork to identify novel therapeutic strategies for CRC patients.

P212

IMMUNOMODULATORY EFFECTS OF HUR INHIBITION IN EGFR-TKI RESISTANT NSCLC CELLS

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BACKGROUND-AIM

Immunotherapy has shown positive results in patients with advanced non-small cell lung cancer (NSCLC), except in those harboring epidermal growth factor receptor (EGFR) mutations. In particular, after resistance to EGFR tyrosine kinase inhibitors (EGFR-TKIs) develops, EGFR-mutant NSCLC patients fail to respond to immune checkpoint inhibitors (ICIs), even in the presence of PD-L1 expression. Acquired resistance to EGFR-TKIs is associated with an immunosuppressive phenotype, including reduced class I HLA antigen expression and increased secretion of IL-6, IL-8, and VEGF. These are targets of the RNA-binding protein HuR, which regulates the stability and translation of bound transcripts. Given HuR's emerging role in immune evasion, we investigated its function in cytokine regulation and class I HLA expression in EGFR-TKI-resistant cells.

METHODS

EGFR-TKI-resistant cell lines (HCC827GR/PC9GR/PC9OR/H1975OR) were generated by treating HCC827/PC9/H1975 cells with Gefitinib or Osimertinib. PC9- and H1975-HuR-KO were generated by CRISPR/Cas9 technology. KH-3 and SRI-42127 commercially available HuR inhibitors were used. Protein expression was analyzed by immunoblotting, confocal microscopy, flow cytometry. Cytokine secretion by ELISA assay and flow cytometry-based multiplex immunoassays.

RESULTS

Confocal microscopy revealed increased cytoplasmic HuR indicative of functional activation in resistant versus parental cells. IL-6 and IL-8 levels were significantly elevated in resistant cells ($p<0.01$) and reduced in HuR-KO lines ($p<0.05$). HuR loss impaired acquisition of resistance to gefitinib and osimertinib and led to a marked (>80%; $p<0.01$) reduction in IL-8 secretion. Pharmacologic inhibition of HuR with SRI-42127 and KH-3 (2.5μM, 24 hours) also decreased cytokine release in both sensitive and resistant cells. Notably, HuR depletion increased class I HLA expression without affecting PD-L1. This upregulation was confirmed following HuR inhibition, particularly in PC9OR and H1975OR cells, suggesting improved antigen presentation.

CONCLUSIONS

Our results indicate that HuR contributes to EGFR-TKIs resistance acquisition and modulates IL-8 and class I HLA expression, which are determinants of poor response to ICIs. These studies support the evaluation of HuR inhibitors to restore the EGFR-TKI responsiveness to immunotherapy.

P213

PHARMACOLOGICAL INHIBITION OF THE RNA-BINDING PROTEIN HUR REDUCES CELL PROLIFERATION AND SURVIVAL IN EGFR-TKI RESISTANT NSCLC CELLS

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BACKGROUND-AIM

Patients with advanced non-small cell lung cancer (NSCLC) harboring EGFR mutations are treated with tyrosine kinase inhibitors (TKIs) like gefitinib or osimertinib. However, most eventually develop resistance, often due to secondary EGFR mutations, alternative receptor activation, or epigenetic changes. Recent evidence highlights the importance of RNA-binding proteins (RBPs) in cancer progression. Among these, HuR (Hu antigen R) is emerging as a key player, as it enhances mRNA stability and translation of genes involved in cell proliferation, evasion of cell death, and drug response making it a promising therapeutic target in EGFR-TKI resistant NSCLC cells.

METHODS

EGFR-TKI-resistant cell lines (HCC827GR/PC9GR/PC9OR/H1975OR) were generated by treating HCC827/PC9/H1975 cells with gefitinib or osimertinib. HuR role was studied using PC9- and H1975-HuR-KO cells generated by CRISPR/Cas9 technology and commercially avaible HuR inhibitors KH-3 and SRI-42127. Cell cycle and apoptosis were analyzed by flow cytometry. Protein expression was analysed by immunoblotting.

RESULTS

In silico analyses revealed elevated HuR mRNA and protein levels in primary lung adenocarcinomas compared to normal tissue, with further increases upon development of EGFR-TKI resistance. Similarly, EGFR-TKI-resistant cell lines displayed higher HuR expression than parental cells. HuR-KO cells showed markedly reduced proliferation, with G0/G1 cell cycle arrest, upregulation of p21 Cip1/Waf1, and downregulation of Cdk2/Cdk6. Pharmacological HuR inhibition with SRI-42127 and KH-3 (2.5 μM, 24h) impaired both G1/S and G2/M transitions in TKI-sensitive and resistant cells, dependently on the method used for cell cycle synchronization (serum deprivation or nocodazole treatment). Regarding cell survival, while HuR gene ablation did not affect cell viability, both HuR inhibitors induced a significant increase of the apoptotic rate in all EGFR-TKI-sensitive and resistant cell lines.

CONCLUSIONS

In conclusion, our findings indicate HuR protein as relevant mechanism and potential target in EGFR-TKI resistant NSCLC given its pro-survival and proliferative functions.

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PLASMA-BASED MIRNOME OF MALE BREAST CANCER CASES FOR THE IDENTIFICATION OF NOVEL MOLECULAR BIOMARKERS ASSOCIATED WITH BRCA STATUS

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BACKGROUND-AIM

Male breast cancer (MBC) is a rare condition, and gaps remain in our understanding of its optimal clinical management. It is well established that pathogenic variants (PVs) in the BRCA genes increase susceptibility to MBC. Patients with BRCA-associated MBC are candidates for personalized treatment approaches, making them an ideal group for investigating diagnostic, preventive, and therapeutic strategies.
Liquid biopsy (LB) has emerged as a minimally invasive tool for detecting molecular biomarkers without requiring traditional tumor biopsies. It provides valuable insights into cancer biology by analyzing circulating cell-free nucleic acids (cfRNA, cfDNA) and proteins. Among cfRNAs, circulating microRNAs (cmiRNAs) have greater stability and higher tissue specificity compared to mRNAs. Thus, cmiRNA profiling in LB is a promising approach for identifying biomarkers relevant to cancer detection and progression.
This study aims to conduct a comprehensive plasma-based profiling of cmiRNAs to identify novel molecular biomarkers associated with MBC. A particular focus is placed on comparing cmiRNA profiles between BRCA PV carriers and non-carriers, to improve early detection and tailoring clinical management.

METHODS

We collected plasma samples from 43 MBC patients (21 BRCA PV carriers and 22 non-carriers). cmiRNAs were extracted using the miRNeasy Serum/Plasma Advanced Kit (Qiagen), and quality and quantity assessments were performed using the Qubit Flex 4.0 fluorometer and the Bioanalyzer Small RNA Kit. miRNome profiling was conducted using the QIAseq miRNA Library Kit (Qiagen), on the Illumina MiSeq i100+ platform. All sequencing runs passed quality control checks, ensuring robust data for analysis.

RESULTS

Differential expression analysis was performed using the Qiagen RNA-seq Analysis Portal to assess the impact of BRCA status on cmiRNA expression profiles. A total of 151 cmiRNAs were initially found to be differentially expressed between BRCA PV carriers and non-carriers. After correction for multiple testing, two cmiRNAs—hsa-miR-320b and hsa-miR-150-5p—remained significantly downregulated in BRCA PV carriers. Both are previously reported in the literature to play roles in breast cancer biology.

CONCLUSIONS

This study provides the first evidence that plasma hsa-miR-320b and hsa-miR-150-5p may serve as potential biomarkers to distinguish MBCs with and without BRCA PV, improving early diagnosis and clinical management of MBCs. Further studies are ongoing to see results in a larger cohort.

P215

THE INTERACTION OF CGAS-STING AND THE DNA DAMAGE RESPONSE PATHWAY IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS: SIGNIFICANCE FOR CHEMOTHERAPY RESISTANCE AND INNOVATIVE THERAPEUTIC APPROACHES

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BACKGROUND-AIM

Acute lymphoblastic leukemia (ALL) is a blood cancer characterized by the accumulation of immature B-/T-cell lymphoid progenitors. Mostly treatments for adult ALL patients use the association of chemotherapy agents however the overall survival rates remain low, as a large percentage of patients relapses or becomes resistant to conventional therapies. Many factors appear to influence the efficiency of the chemotherapy regimen, such as DNA damage repair (DDR)mechanisms and immunological pathways such as the cGAS-STING pathway.

METHODS

RS411, Reh, KOPN-8, and HAL-01 ALL cells were made resistant through treatment with vincristine and doxorubicin. Primary cells were obtained through collaboration with the University of Bergen. The evaluation of gene expression of DDR and STING factors was performed using qPCR. For drugs screening, we selected as PARP1 inhibitors (Talazoparib) and as STING agonists ENPP1-IN-1. The assessment of cell viability and proliferation was performed using RealTime-Glo and for combination index assay using MTS assay. Cell apoptosis was performed using Annexin V/Pi staining kit. The evaluation of cellular morphology was assessed using a May-Grunwald/Giemsa stain.

RESULTS

We performed RT-PCR to assess the baseline expression levels of key DDR-related and cGAS-STING pathway genes in our parental and chemoresistant ALL cell lines (here after ChemoR cells). We found an upregulation of different genes of DDR systems and a simultaneous downregulation of different cGAS-STING pathway genes. Based on this findings, we evaluated the response of the cells to a PARP1 inhibitor (Talazoparib) and cGAS-STING agonists (e.i. ENPP1 inhibitor-1). We found that the inhibition of PARP1 or the inhibition of ENPP1 significantly reduced the cell viability of both parental and ChemoR cells. Interestingly, we found that the combination of subtoxic concentrations of talazoparib and ENPP1 inhibitor after 5 days of treatment resulted in a synergistic induction of apoptosis of our ChemoR cells. We also performed an evaluation of morphology trough MayGrunwald/Giemsa staining and we found a significant increase of polynucleated cells and an overall increase of cell volume in the samples treated with the combination.

CONCLUSIONS

In the present study, we showed that prolonged inhibition of PARP1 and ENPP1 leads to increased cell death of chemoresistant cell. We hypothesized that this may be related to a synergism of these two drugs wich leads to a mitotic catastrophe through activation of the cGAS-Sting pathway.

P216

A DUAL-FLUORESCENCE CD63 REPORTER SYSTEM AS A TOOL TO MONITOR THE RELEASE OF EXTRACELLULAR VESICLES IN SENESCENT CARDIOMYOCYTES

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BACKGROUND-AIM

Cellular senescence is increasingly recognized as a key contributor to cardiovascular disease (CVD), although its precise role remains unclear. Senescent cells promote disease progression through irreversible cell cycle arrest and the secretion of pro-inflammatory factors, including extracellular vesicles (EVs). This study used a dual-fluorescent CD63-based reporter system as a powerful, real-time tool for high-resolution monitoring of EVs secretion, overcoming traditional endpoint assays.

METHODS

The system was applied in a density-induced senescent cardiomyocyte (HL-1) model, providing a versatile platform to explore EV-mediated intercellular communication during cardiac aging. HL-1 cells were transduced with a lentiviral vector encoding CD63 fused to a pH-sensitive pHluorin (GFP) and a mScarlet (RFP) protein. After blasticidin selection, senescence was induced by prolonged high-density culture.

RESULTS

Senescence was confirmed by increased levels of key markers, including β-galactosidase activity, p21 expression, and γ-H2AX accumulation. Fluorescent EVs were isolated from density-induced senescent HL-1 cells and analyzed via ImageStream flow cytometry combined with MemGLOw membrane labeling, enabling high-resolution, quantitative characterization. A significant increase in EVs secretion, normalized to cell number, was observed in high-density conditions, correlating with the senescent phenotype.

CONCLUSIONS

This density-induced in vitro model highlights the link between senescence and EVs release in cardiomyocytes. Moreover, using fluorescently labeled EVs offers an innovative, quantitative approach to monitor vesicle secretion, providing new insights into intercellular communication during cardiac aging and the development of age-related CVD.

P217

NF-κB AXIS IN RETINAL GLIAL CELLS: IMPLICATIONS IN THE GLAUCOMA PATHOLOGY

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BACKGROUND-AIM

Glaucoma is a neurodegenerative disease characterized by the progressive loss of retinal ganglion cells (RGCs) and optic nerve damage that reflect in vision loss. Mechanical and vascular factors are believed to contribute to the etiology of glaucoma, however pathogenic mechanisms are complex and not yet fully understood. Neuroinflammation plays a pivotal role in both the development and the progression of the disease, and NF-κB, a key transcriptional driver of inflammation, is considered to be crucial in glaucomatous degeneration. However, many questions are still unsolved such as to clarify how glial cells in the mammalian retina lead and sustain the glaucoma dysfunction.
Therefore, one of the aims is to identify the role of NF-κB transcriptional targets in the established glaucoma model.

METHODS

We used a technology able to simulate in vitro an increase of hydrostatic pressure (Live Flow+Live PA (IVTech)) on the Retinal glial Müller cells. The rMC1 cell line was cultured under stress conditions, alone or in combination with high hydrostatic pressure, simulating the synergistic effect that occurs in the pathogenesis of glaucoma. Molecular analysis (RT-qPCR and Western blot analysis) has been performed to clarify the involvement of the NF-κB pathway in the glaucoma pathogenesis.

RESULTS

Our data demonstrated that Live Flow+Live PA technology induced higher cellular distress compared to the controls as well as the NF-κB/p65 activation suggesting the involvement of NF-κB pathway in the early phase of the glaucoma pathogenesis. Analysis of transcriptional targets of NF-κB are ongoing.

CONCLUSIONS

Understanding the role of NF-κB pathway in the pathology of glaucoma is important to identify specific transcriptional targets of NF-κB able to modulate glaucoma-related neuroinflammation and anti-apoptotic functions. The final goal is to potentially test specific compounds able to counteract neuroinflammation interfering with the NF-κB axis.

P218

RETINAL MüLLER CELLS RESPONSE IN GLAUCOMA: INSIGHT TO MOLECULAR MECHANISMS USING 3D BIOPRINTING APPROACH

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BACKGROUND-AIM

Glaucoma represents the second cause of blindness in the world. Despite it is known that the main cause of neuronal injury and retinal degeneration is an elevated intraocular pressure (IOP), molecular mechanisms involved in development and progression of glaucoma are poorly understood. Moreover, the structure of retina includes different types of cells that could respond to IOP both promoting or countering RGCs degeneration. In this context, Müller cells in neuroretinal layer play a relevant role, supporting retinal ganglion cells but also contributing to pathophysiology of glaucoma. To study such a complex system and elucidate the molecular mechanisms underlying the pathogenesis of glaucoma, we generated an advanced model of disease using 3D bioprinting technology. We also evaluated a neurotrophic compound (NTRC) as potential therapeutical agent.

METHODS

To mimic neuroretinal and retinal pigment epithelium layers we designed a co-culture system constituted by bioink-embedded Rat Retinal Müller Cells (rMC-1) on top of ARPE19 cell monolayer. 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.

RESULTS

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. 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.

CONCLUSIONS

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

P219

FUNCTIONAL INTERACTION BETWEEN CELLULAR COMPONENTS AND SOLUBLE FACTORS FOR BONE MARROW AUTOLOGOUS CONCENTRATE THERAPY IN CRITICAL LIMB ISCHEMIA

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BACKGROUND-AIM

Critical Limb Ischemia (CLI) represents the most severe stage of peripheral arterial disease, characterized by resting pain and ischemic ulcers. Recent therapeutic approaches based on bone marrow autologous concentrates (BMAC) have shown promising results for CLI treatment. In this study, we evaluated the functional interaction between soluble factors and cellular components in BMAC, by correlating these parameters with clinical outcomes at 30 days from infiltration.

METHODS

22 patients with CLI were enrolled and underwent clinical, molecular and cellular characterization at baseline (T0). Peripheral Blood (PB) and Bone Marrow (BM) samples, from which the autologous concentrate (BMAC) was obtained, were collected and analyzed by cytofluorimetry screening and ELISA-multiplex assay for the quantification of 27 cytokines, chemokines and growth factors. Data were then correlated with clinical outcome parameters such as treadmill test (TT) and transcutaneous partial pressure of Oxygen ($TcpO_2$).

RESULTS

BMAC treatment led to an amelioration of clinical outcome, with significant increases for both TT and $TcpO_2$ at 30 days (p-value <0.001). Baseline evaluation highlighted a negative correlation between $TcpO_2$ and inflammatory markers IL-2 and TNFα in all three matrices (PB, r: -0.627 and -0.528; BM, r: -0.449 and -0.507; BMAC, r: -0.560 and -0.504). Evaluation of both cellular and soluble factors screened at T0 and clinical outcome parameters showed that, in PB, higher levels of IL-12 (OR: 1.31) and Mesenchymal Stem Cells (MSC) (OR: 1.28) were associated with a higher increase in $TcpO_2$. In BMAC, the increase in TT was negatively associated with IL-2 levels (OR: 0.79), while a better response for $TcpO_2$ was associated with lower IL-9 levels (OR: 0.77) and with higher concentrations of CD34+ (OR: 1.81) and CD34/45 (OR: 3.06) cellular subpopulations.

CONCLUSIONS

BMAC confirms its potential as a promising therapeutic option for CLI, improving key clinical outcome parameters in a short time frame. Both systemic and BMAC-specific levels of IL-2 and TNFα showed a negative correlation with $TcpO_2$. Moreover, a specific array of both cellular and soluble factors in BMAC showed a potential predictive role in CLI therapy.

P220

ENDOTHELIAL ACTIVATION BY HEMOZOIN AND ANTIMALARIALS: IMPLICATIONS FOR SEVERE MALARIA

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BACKGROUND-AIM

Malaria is a parasitic disease causing millions of cases every year. A key aspect in the pathogenesis of severe malaria is endothelial activation, which facilitates the adhesion of infected erythrocytes to the vascular wall, contributing to inflammatory and thrombotic complications. Parasite-derived components such as hemozoin (HZ) contribute to endothelial activation. Hemozoin produced by parasites during intra-erythrocytic stages, is released into the circulation of malaria infected patients. While HZ is known to activate phagocytic cells such as monocytes and macrophages, its role in endothelial activation is less well understood. This study aimed to evaluate the release of CXCL8 and von Willebrand factor (vWF) from endothelial cells stimulated with HZ. Additionally, given that antimalarial therapy relies mainly on artemisinin derivatives, the effect of dihydroartemisinin (DHA) in this model was assessed.

METHODS

HMEC-1 and HuLEC-5a microvascular endothelial cells from dermis and lung, respectively were used. Cells were treated with HZ (20-10-5 µg/mL), either alone or in the presence of DHA (1 µM) for 2 or 24 hours. HZ was isolated from P. falciparum cultures by Percoll gradient centrifugation. In some experiments, cells were treated with fibrinogen (200-µg/mL) a plasma protein known to be bound to the HZ. Gene expression and protein secretion of CXCL-8 were evaluated by Real-Time PCR and ELISA, respectively. Release of vWF was evaluated by ELISA.

RESULTS

In both HMEC-1 and HuLEC-5a, HZ increased gene expression and protein secretion of CXCL8. Increased levels of CXCL-8 were observed also at 2 hours post-treatment suggesting that HZ triggers an early release of the chemokine from preformed stores within Weibel-Palade bodies. DHA, one of the most used antimalarial drug, exerted an inhibitory effect on CXCL8 expression and production by interfering with the de novo synthesis of the chemokine, but did not affect its early release from Weibel-Palade bodies. Fibrinogen increased CXCL8 production, particularly after 24 hours in HuLEC-5a cells. Von Willebrand factor release was significantly increased following HZ stimulation at both 2 and 24 hours confirming HZ ability to induce rapid and effective endothelial activation.

CONCLUSIONS

These data suggest that hemozoin-activated endothelium may contribute to the increased plasma levels of vWF and CXCL-8 observed in complicated malaria.

P221

ELUCIDATING THE COMPLEXITY OF CHEMORESISTANCE IN ACUTE LYMPHOBLASTIC LEUKEMIA: DNA DAMAGE RESPONSE AND BEYOND

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BACKGROUND-AIM

Acute lymphoblastic leukemia (ALL) remains a challenging malignancy, with chemoresistance contributing significantly to treatment failure and relapse. The biological mechanisms underlying chemoresistance development are mostly unknown. This study aimed to investigate the biology of chemoresistant ALL cells by generating and characterizing chemoresistant ALL cell lines from molecular and functional perspectives. The goal was to identify potential therapeutic strategies to overcome chemoresistance.

METHODS

We established ten chemoresistant ALL cell lines (ChemoR): four resistant to vincristine, four to doxorubicin, and two to both agents. Chemoresistance was induced through stepwise drug exposure, and resistance levels were confirmed by in vitro viability, apoptosis and clonogenic assays. Multidrug resistance (MDR) transporter expression was assessed by flow cytometry. DNA damage response (DDR) pathway involvement was analyzed at gene and protein level. Transcriptomic profiling and functional assays, including apoptosis induction, cell cycle profiling, and clonogenic survival, were performed to characterize resistance mechanisms of ChemoR cells. Combination index analysis evaluated the efficacy of DDR inhibitors in combination with chemotherapeutics or selective inhibitors. The best combinations were tested ex-vivo on peripheral blood mononuclear cells (PBMC) from healthy donors and on ALL relapsed patients.

RESULTS

ChemoR ALL cells showed higher IC50 values, apoptosis resistance and inactivation of cell cycle checkpoints with no variation in MDR transporter levels. DDR pathway analysis revealed differential expression of key kinases (ATR, ATM, CHK1 and WEE1), pointing out their role in sustaining chemoresistance. ChemoR cells exhibited enhanced capacity for DNA damage repair upon exposure to H_2O_2 or ionising radiation. Transcriptomic analysis identified altered pathways shared by these models, including gene regulation, drug metabolism and the innate immune response. In order to overcome chemoresistance, the efficacy of drug combinations of DDR inhibitors with vincristine or doxorubicin was analysed. Combinations with ATM and PARP1 inhibitors, were also tested ex vivo on PBMC of ALL relapsed patients, showing promising translational results.

CONCLUSIONS

The study provides critical insights into ALL chemoresistance mechanisms. Our findings suggest DDR alterations contribute significantly to resistance and that ATM and PARP1 inhibition may offer a promising therapeutic strategy.

P222

PERITUMORAL ADIPOSE TISSUE INTERSTITIAL FLUIDS: A NEW SOURCE FOR BIOMARKER DISCOVERY IN BREAST CANCER?

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BACKGROUND-AIM

The tumor microenvironment (TME) in breast cancer (BC) has important functions in tumor behaviour and treatment response, making its pathologic assessment critical for the disease management. The major component of the TME in mammary gland is represented by adipose tissue (AT). Different studies have shown that AT may contribute to tumor phenotypes by either acting as an energy reservoir for embedded cancer cells and through the secretion of signaling molecules. Interstitial fluids (IF) represent the interface between circulating bodily fluids and intracellular fluids. IF is a real reservoir of cytokines, chemokines and growth factors released by cancer and TME cells and thus may represent an optimal source for new biomarker discovery.

METHODS

Peritumoral (PT-at the margin of tumor) and distal (DT- about 5 cm from tumor) AT biopsies from N= 25 patients with estrogen receptor positive BC have been obtained. IF have been collected and tested for the concentration of a panel of 27 molecules by using a Multiplexed immunoassay on a Luminex® 200™ System.

RESULTS

PT-IF displayed significantly higher amount of APRIL, CCL-2 (MCP-1), CCL-4 (MIP1-β), IL-6, IL-8, IL-10, CCL-3 (MIP-1α), CCL-11 (eotaxin), CXCL-10 (IP-10), IFN-γ, TNF-α, IL-1RA, IL-6 Ra, CRIPTO, FURIN, u-PA, BAFF, CCL5 (RANTES) and FAP, compared to DT-IF. Correlation matrices revealed a signature of molecules characteristic of PT-IF different from that observed in DT-IF. In addition, in PT-IF, CCL-2 concentration positively correlated with patient age, while u-PA, BAFF, CD44, IL-6, APRIL, CCL-2, CCL-4, IFN-γ, IL-8 and TNF-α positively correlated with patient glycaemia. An inverse correlation was observed for CCL5 with tumor dimension and for CCL-2, IL-6 and CD44 with tumoral expression of Progesteron receptor. Interestingly, PT-IF α-synuclein (r: 0.38; 95% CI: 0.02 to 0.68) and total inhibin (r: -0,44: 95% CI: -0.71 to -0.04) significantly correlated with the tumoral marker ki-67.

CONCLUSIONS

BC and surrounding AT interact through the release of multiple factors needed for cancer dissemination and resistance to drugs. PT-AT IF factors may represent a novel signature of cancer aggressiveness.

P223

PRDM2 INTERSECTS THE MEVALONATE AND EGFR PATHWAY: A NEW TARGET FOR THE COLORECTAL CANCER TREATMENT

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BACKGROUND-AIM

The Mevalonate (MVA) pathway plays a key role in cell survival and growth being involved in cholesterol biosynthesis and protein prenylation. N6-isopentenyladenosine (IPA) and its analog N6-benzyladenosine (N6BA), are modified nucleosides with antitumor activity in cancer, including colorectal cancer (CRC), able to inhibit DNA methyltransferases (DNMTs) and farnesyl diphosphate synthase (FDPS), a key enzyme of the MVA pathway. RIZ1 and RIZ2, encoded by the PRDM2/RIZ gene, display tumor-suppressive and oncogenic features, respectively, and are deregulated in cancer. An imbalance favoring the oncogenic isoform RIZ2 over the tumor suppressor RIZ1 is often observed in many cancer types, as CRC. We previously demonstrated that RIZ2 overexpression deregulates proteins involved in MVA and EGF signaling. Here, the efficacy of IPA and N6BA in modulating EGFR/PRDM2/MVA crosstalk was investigated.

METHODS

In DLD1 cells overexpressing RIZ2 (DLD1_hRIZ2), the effect of IPA and N6BA on RIZ1/RIZ2 expression level was evaluated through RT-PCR analysis. BrdU assay was performed to analyze the efficacy of IPA or N6BA combined with the EGFR-TKI, Erlotinib. EGFR pathway activation was assessed by Western blot analysis, in presence or absence of EGF. Cross-link of membrane protein with BS3 crosslinker and confocal microscopy analysis were performed to study the effect on EGFR dynamic.

RESULTS

In DLD1_hRIZ2 cells IPA and N6-BA shifted the RIZ1/RIZ2 balance in favor of the tumor suppressor RIZ1 by inducing its expression.
Prolonged exposure to both IPA and N6BA showed an antiproliferative effect coupled with EGFR signaling inhibition, also in DLD1_hRIZ2 cells, characterized by EGFR hyperactivity. Moreover, pre-treatment with IPA and N6-BA enhanced the sensitivity to Erlotinib.
Interestingly, both IPA and N6BA caused an early EGFR level increase that is however uncoupled with the downstream ERK activation. Confocal microscopy analysis suggested that IPA and N6BA induced EGFR coalescence through plasma membrane. In addition, they affect the dynamic of EGFR activation, modulating active dimers within the membrane.

CONCLUSIONS

These findings support the therapeutic potential of IPA and N6-BA. By modulating the RIZ1/RIZ2 balance and interfering with EGFR/MVA signaling crosstalk, these compounds may represent promising candidates for personalized therapeutic strategies based on the molecular features of individual tumors.

P224

ROLE OF MAPT/TAU IN PROMOTING ENHANCED ONCOGENIC PHENOTYPE IN NEUROBLASTOMA SH-SY5Y CELLS

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BACKGROUND-AIM

Tau protein, encoded by MAPT gene, belongs to the family of Microtubules-Associated Proteins (MAPs). Through alternative splicing, MAPT gene gives rise to six isoforms that are differentially expressed during embryonic development and post-natal period. In humans, Tau protein has been mainly investigated in tauopathies as Alzheimer's disease, in which Tau phosphorylated forms aggregate and deposit leading to neuronal degeneration. However, Tau biology should be further investigated since several tumors express differential levels of MAPT, including neuroblastoma (NB), a highly heterogenous extracranial tumor with neuroendocrine origin. In particular, NB SH-SY5Y cells represent a plastic in vitro model to study cell differentiation and acquired therapeutic resistance.
Our study aims to provide new evidence of the pro-tumorigenic and anti-apoptotic potential of Tau protein in promoting an aggressive tumor phenotype characterized by enhanced cell proliferative and migratory capability.

METHODS

SH-SY5Y cells were used as reference NB cell model; retinoic acid (RA) and sodium butyrate (NaB) were adopted as differentiation agents. MAPT expression levels were analyzed by PCR and western blot. RNAi silencing was performed to transiently knock out MAPT expression.

RESULTS

MAPT expression is confirmed in NB SH-SY5Y cell line as a main predominant embryonal isoform. Upon RA administration, Tau protein is down-expressed followed by reduced expression levels of vimentin and ß-catenin markers, resulting in a post-mitotic tumor phenotype characterized by cells with "neuronal"-like morphology. On the contrary, NaB can promote the up-regulation of Tau protein and enhanced vimentin and ß-catenin expression levels, leading the transition towards a more aggressive tumor phenotype. These data are further supported by a reduced cell viability and migration in RA-treated cells compared to NaB-treated cells. Gene silencing evidences that tumor phenotypical aggressiveness is largely abolished switching off MAPT expression, also reducing tumor resistance to genotoxic agents, as doxorubicin.

CONCLUSIONS

These results show that highly-expressing-Tau tumors are characterized by enhanced phenotypical aggressiveness and may potentially show acquired resistances to chemotherapeutic agents. Therefore, it may be interesting to further characterize Tau protein interactions with other oncogenic molecular markers, including vimentin and ß-catenin, to potentially open new therapeutical routes.

P225

MIR-200C SYNERGIZES WITH TRASTUZUMAB-LOADED GOLD NANOPARTICLES TO OVERCOME RESISTANCE IN OVARIAN CANCER CELLS

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BACKGROUND-AIM

Trastuzumab (TZ) resistance remains a significant challenge in the treatment of human epidermal growth factor receptor 2 (HER2)-positive epithelial ovarian cancer (EOC), necessitating novel therapeutic strategies to improve treatment efficacy. Functionalized gold nanoparticles (AuNPs) constitute a promising platform for drug delivery and the ability to enhance tumor targeting via the enhanced permeability and retention (EPR) effect. miR-200c, a well-established tumor suppressor microRNA (miRNA), plays a crucial role in inhibiting epithelial-mesenchymal transition (EMT).

However, its role in modulating the human epidermal growth factor receptor 2 (HER2) signaling pathways and sensitizing ovarian cancer cells to TZ remains largely unexplored. Here, we investigate for the first time the combinatorial effect of miR-200c and thiol-functionalized AuNPs (< 10 nm) loaded with TZ (AuNPs-TZ) in overcoming TZ resistance and enhancing treatment efficacy in ovarian cancer cells.

METHODS

Thiol-functionalized AuNPs were synthesized and noncovalently loaded with TZ. SKOV3 ovarian cancer cells were transfected with miR-200c and treated with AuNPs, TZ, or AuNPs-TZ. Cell viability and apoptosis were assessed using MTT assays and flow cytometry, respectively. Protein expression was analyzed by Western blot, and nanoparticle localization was evaluated via transmission electron microscopy (TEM).

RESULTS

Pristine AuNPs were not cytotoxic, confirming their biocompatibility as a nanocarrier for TZ delivery. AuNPs were loaded noncovalently with TZ and maintained colloidal stability to prevent aggregation while facilitating effective cellular uptake. Treatment of ovarian cancer cells overexpressing miR-200c with AuNPs-TZ significantly reduced cell viability and increased apoptosis. Immunoblot analysis showed a reduction of phosphorylated HER2 and downstream Kirsten Rat Sarcoma Virus (KRAS) signaling. Furthermore, TEM demonstrated morphological changes in miR-200c-transfected ovarian cancer cells and confirmed the localization of AuNPs carrying TZs on the cell membrane and in the cytoplasm.

CONCLUSIONS

These findings highlight the potential of AuNPs-TZ delivery combined with miR-200c as a promising therapeutic strategy to improve the response of HER2-positive EOC to TZ treatment. These results imply the need to further develop AuNP/miRNA-based combinatorial therapies as a viable nanomedicine approach for drug-resistant cancers.

P226

IN VITRO MODEL BASED ON A TRANSGENIC LEISHMANIA STRAIN TO INVESTIGATE HOST-PARASITE INTERACTIONS AND TO IDENTIFY NEW DRUGS

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BACKGROUND-AIM

Leishmaniasis is a Neglected Tropical Disease caused by protozoa of the Leishmania genus. No human vaccine is available, and current treatments are limited, often toxic, and compromised by drug resistance. Thus, identifying new therapeutics and developing faster, more accurate drug screening methods is a priority. The parasite alternates between promastigotes in the insect vector and intracellular amastigotes in host macrophages, key cells in both parasite survival and clearance. Studying macrophage–Leishmania interactions is essential for understanding disease pathogenesis and advancing therapeutic strategies.

METHODS

A transgenic L.infantum line stably expressing the bioluminescent marker PpyRE9H fused to the fluorescent reporter tdTomato (PpytdT+Li) (Calvo-Alvarez et al., 2018) was generated. Murine bone marrow derived macrophages (BMDM), human monocytic THP-1 cells di[erentiated into macrophages (dTHP-1), and primary monocyte derived macrophages from peripheral blood mononuclear cells (MDM) were infected with transgenic or wild type L. infantum promatigotes. The percentage of infection and the activity of known drugs were evaluated by fluorescence microscopy, by using a Muse® Cell Analyzer and a microplate fluorescence/luminescence reader Synergy4®. Results were compared to those obtained with the Giemsa staining reference method.

RESULTS

The infection index of macrophages treated with transgenic and wild-type Leishmania are comparable. Data obtained using MUSE or fluorescence microscopy are consistent with those obtained using the standard Giemsa staining method. For the chemosensitivity assay the IC50 values of known antileishmanial agents were evaluated using a new bioluminescence and fluorescence microplate assay. The results showed that the IC50 values obtained with these assays and transgenic Leishmania are comparable to those obtained using the conventional Giemsa staining method

CONCLUSIONS

Further studies are needed to validate these methods using a broader library of compounds with known IC50 values. Nonetheless, these assays appear promising as alternatives to the conventional Giemsa- based method.

P227

MAPT/TAU AS A POTENTIAL PROGNOSTIC BIOMARKER IN PROSTATE CANCER: EVIDENCE FROM TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS

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BACKGROUND-AIM

Prostate cancer (PCa) is a leading cause of cancer-related death in men and is marked by significant biological heterogeneity, making it difficult to predict disease progression and treatment response. Recent studies have linked the expression of Tau protein, traditionally associated with neurodegenerative diseases, to drug resistance in various cancers. Tau, encoded by the MAPT gene, exists in six isoforms generated through alternative splicing, each with distinct spatial and temporal expression patterns. This study aims to investigate the expression and distribution of Tau isoforms in prostate cancer cell lines representing different stages of tumor progression. The goal is to explore potential correlations between Tau isoform profiles, clinical prognosis, and therapeutic responsiveness.

METHODS

Epithelial prostate cell lines WPE-1 NB-26 and RWPE-1, and prostate cancer cell lines LNCaP (androgen-sensitive, low aggressiveness), DU145 and PC3 (androgen-independent, high aggressiveness) were used as reference models. Total and isoform-specific expression profiles of Tau protein were assessed by immunoblotting and PCR. Tau protein was then silenced using RNA interference, followed by treatment with doxorubicin to induce DNA damage and apoptosis.

RESULTS

Tau protein expression increases progressively from normal epithelial cells to metastatic PCa cell lines. Transcriptomic analysis revealed a shift in N-terminal isoforms: the 0N isoform is predominant in normal and less aggressive tumor cells, while 1N and 2N isoforms are enriched in highly aggressive tumor cells. The ratio of 3R to 4R isoforms remains balanced. Higher Tau expression correlates with tumor increased aggressiveness and nuclear localization, suggesting a non-canonical role. To investigate Tau's potential function in DNA protection, cells were silenced for Tau and treated with doxorubicin. Tau-silenced cells showed greater sensitivity to the drug, indicating that Tau may support cell survival under genotoxic stress.

CONCLUSIONS

Tau may promote PCa cell survival and chemoresistance, making it a potential prognostic biomarker and therapeutic target. Its overexpression, shift to 1N/2N isoforms, and nuclear localization in aggressive cell lines support this role. Clinical studies are needed to confirm correlations with tumor grade.

P228

AGE-RELATED TRENDS OF SERUM NEUROFILAMENT LIGHT CHAIN IN HOSPITALIZED AND COMMUNITY-DWELLING COHORTS AND ITS PROGNOSTIC VALUE FOR MORTALITY

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BACKGROUND-AIM

Serum neurofilament light chain (sNfL) is an established biomarker of neuroaxonal damage with growing prognostic relevance across neurological and systemic conditions. However, its role in predicting outcomes in older adults—particularly in relation to cognitive status and renal function—remains insufficiently studied.

METHODS

We analyzed data from 1,052 hospitalized patients aged ≥65 years enrolled in the Report-AGE project (INRCA, Ancona) and 915 community-dwelling older adults from two independent cohorts in Bologna and Milan. All participants underwent comprehensive geriatric assessment and blood sampling for sNfL measurement alongside routine laboratory tests. The study aimed to evaluate age-related patterns of sNfL and its association with sex, cognitive status, kidney function, and 10-year mortality.

RESULTS

sNfL levels increased significantly with age across all groups, with hospitalized patients exhibiting the steepest rise compared to the two community-dwelling cohorts, which showed similar trends. Among hospitalized patients, those who died during follow-up had significantly higher sNfL levels than survivors. Elevated sNfL was also associated with higher comorbidity, frailty, and reduced kidney function. When stratified by cognitive status and sNfL categories (<40, 40–66, ≥67 pg/mL), higher sNfL levels correlated with advanced age, cognitive impairment, lower eGFR, and increased long-term mortality. Patients with sNfL ≥67 pg/mL had a twofold higher risk of death compared to those with sNfL <40 pg/mL, an association that persisted after adjustment for demographic, clinical, and laboratory variables. Spline modeling showed that the predictive power of sNfL diminished in patients with severe renal impairment (eGFR <30 mL/min) but remained consistent across eGFR levels in cognitively impaired individuals.

CONCLUSIONS

In conclusion, sNfL is a robust biomarker of long-term mortality in older hospitalized adults. Its prognostic significance is influenced by cognitive and renal function, underscoring the importance of integrating multiple biomarkers for risk stratification in geriatric populations.

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

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BACKGROUND-AIM

Carboxylesterase 1 (CES1) has been identified as an essential NF-κB-regulated lipase that regulates metabolic adaptation and survival in colorectal cancer (CRC). Data generated in our laboratory underline the importance of the CES1-dependent metabolic mechanism also in epithelial ovarian cancer (EOC), suggesting that CES1 could be a promising target to counteract the growth of these tumours. 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. Our analysis of scRNA data from patients with CRC and EOC indicate that CES1 is highly expressed in specific subsets of tumor-associated macrophages (TAMs) associated with worse prognosis.
These data suggest that CES1 may play a role in TME-associated cells, contributing to the establishment of an immunosuppressive niche that promotes tumor aggressiveness. Therefore, we foresee that targeting CES1 in both cancer cells and TME-associated cells may represent a promising therapeutic target to counteract tumor growth.

METHODS

We established a 3D co-culture model between macrophages generated from PMA-treated THP-1 monocytes - either wild type or knockout for CES1 - and ovarian cancer cells. Spheroids were generated using BIOFLOAT™ 96-well plates and cultured under basal and energetic stress conditions. We monitored spheroid growth and cell viability by CellTiter-Glo® 3.0 assay and CellTrace™ CFSE Cell Proliferation Kit. We also examined whether the absence of CES1 could affect the expression of genes associated with immunosuppression, angiogenesis and macrophage polarization.

RESULTS

These 3D co-culture models revealed that the presence of myeloid cells enhances EOC spheroid growth. Notably, macrophage-specific CES1 silencing abolished this pro-proliferative effect on cancer cell spheroids, suggesting that CES1 depletion may reprogram macrophages towards an anti-tumor phenotype. Preliminary data obtained from differential gene expression analysis between wild type or knockout CES1 macrophages seem to confirm this hypothesis.

CONCLUSIONS

CES1 sustains tumor growth by promoting a protumoral macrophage phenotype. Due to the double role of CES1 in both EOC cells and TME-based cells, targeting this lipase could afford a dual benefit in the management of EOC.

P230

IN VITRO MODEL OF CHOLANGIOCYTE-ENDOTHELIAL CELL INTERACTION: EXPLORING THE INFLAMMATORY BASIS OF THEIR CROSSTALK

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BACKGROUND-AIM

Primary sclerosing cholangitis (PSC) is a rare liver disease characterized by chronic bile duct inflammation. Cholangiocytes actively contribute to disease progression by releasing pro-inflammatory signals that recruit immune cells and neighboring cholangiocytes. Chronic injury may also induce cholangiocyte senescence, associated with a senescence-associated secretory phenotype (SASP) that amplifies inflammation and tissue damage. Similar features have been observed in primary biliary cholangitis (PBC), where senescent cholangiocytes correlate with disease severity. Cellular senescence is increasingly recognized as a driver of chronic inflammation and tissue dysfunction in cholestatic liver diseases.This study investigates the molecular crosstalk between cholangiocytes and endothelial cells, specifically young and senescent HUVECs, to evaluate their contribution to inflammation and senescence in the context of PSC and PBC.

METHODS

yHUVEC and senHUVEC endothelial cells were co-cultured with primary cholangiocytes (Col) for 24 and 48 hours. Each cell type was maintained in its specific medium to avoid medium-induced crosstalk effects, and no medium changes were made during the co-culture. Transwell inserts with 0.4 µm pores were used, according to the literature. Additionally, an in vitro cholangiopathy-like model is being developed by treating normal cholangiocytes with different concentrations of the toxic bile acid GCDC.

RESULTS

Cholangiocytes showed increased mRNA expression of IL-1β and IL-8 when co-cultured with both yHUVEC and senHUVEC. This inflammatory response may result from soluble factors (yHUVEC) or SASP-mediated bystander effects (senHUVEC). IL-6 expression followed a similar pattern, with lower levels at 24h and increased levels at 48h. No significant changes in senescence markers p16 and p21 were observed.

CONCLUSIONS

Endothelial–cholangiocyte crosstalk promotes an inflammatory response, especially in the presence of senescent endothelial cells. This mechanism may contribute to the progression and chronic state of cholangiopathies such as PSC and PBC. The co-culture model and GCDC treatment provide a valuable in vitro system to investigate underlying disease mechanisms where access to patient tissue is limited.

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P231

MACROPHAGES-LEISHMANIA INFANTUM INTERACTIONS: HOW THE PRODUCTION OF MICROBIOCIDAL RADICALS CHANGES UNDER HYPOXIA

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BACKGROUND-AIM

Leishmaniasis is a neglected tropical disease caused by the intracellular protozoa Leishmania. Macrophages are the primary resident cells for the parasites, contributing to the fate of infection by allowing parasite replication. However, macrophages produce nitric oxide (NO) and reactive oxygen species (ROS), which are responsible for the killing of parasites. During Leishmania infection the tissues are inflamed and characterized by lower oxygen tension (hypoxia), but the response of Leishmania-infected macrophages in a hypoxic environment has not been studied. The aim of this work is to investigate the killing mechanisms in an in vitro model of Leishmania-infected macrophages, in normoxia or hypoxia.

METHODS

Murine bone marrow-derived macrophages (BMDM) were infected with Leishmania infantum (L.i.) MHOM/TN/80/IPT1 promastigotes (MOI 10), followed by treatment with IFN-γ+LPS or IL-4+IL-13 to induce a proinflammatory or anti-inflammatory phenotype, respectively. Nitric Oxide Synthase 2 (NOS2) gene expression was evaluated by Real-Time PCR; NO levels in cell supernatants were quantified by Griess assay. ROS production was evaluated using the fluorescence Oxidative Stress commercial kit and the Muse® Cell Analyzer. All the experiments were performed in hypoxia (1% O_2, 5% CO_2) and compared to normoxia (standard culture conditions).

RESULTS

In normoxic conditions, L.i. infection significantly reduced NO levels induced by IFN-γ+LPS. On the other side, infection increased ROS production in both proinflammatory and anti-inflammatory-induced macrophages. In hypoxia, NO production was completely abolished in all the conditions tested, although NOS2 expression was induced. ROS production was increased compared to normoxia. Infection with L.i. reduced ROS production, only in proinflammatory-induced macrophages.

CONCLUSIONS

Under hypoxia, BMDM seemed to counterbalance their inability to produce NO by producing higher levels of ROS. A hypothesis to be tested is that in response to microbiocidal radicals production by macrophages, the parasites try to escape the host defence by reducing NO in normoxia and ROS in hypoxia. A better comprehension of the interplay between Leishmania and macrophages in a hypoxic environment is crucial for a better knowledge of the pathogenesis of leishmaniasis, also in the perspective of new possible treatments.

P232

TARGETING CARBOXYLESTERASE 1 (CES1) TO OVERCOME METABOLIC ADAPTATION IN ACUTE MYELOID LEUKEMIA

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BACKGROUND-AIM

Acute Myeloid Leukemia (AML) exhibits profound metabolic plasticity that enables leukemic cells to survive and proliferate even in nutrient-depleted environments. AML cells rely on oxidative phosphorylation (OXPHOS) and reprogrammed lipid metabolism to meet their high energy demands and sustain survival under stress. This metabolic flexibility not only supports proliferation but also contributes to treatment resistance. Carboxylesterase 1 (CES1) has been identified as a key enzyme in regulating lipid catabolism, thus facilitating energy production under stress conditions. This study aims to evaluate the role and functional relevance of CES1 in AML.

METHODS

Publicly available AML patient datasets were analyzed for CES1 expression. A panel of AML cell lines was tested for CES1 levels both in basal (BC) and energy stress conditions (ES). CES1 expression was measured by Western blot and qPCR. Metabolic phenotype and survival, under BC and ES, with or without CES1 inhibitor, were evaluated on the AML cell lines by Seahorse XFe96, Western Blot, and viability assay.

RESULTS

Our findings indicate that CES1 plays a critical role in sustaining metabolic adaptation in AML cells, particularly under nutrient-depleted conditions, driving the metabolic switch from glycolysis to oxidative phosphorylation (OXPHOS). Pharmacological CES1 blockade with the commercially available inhibitor GR-148672X resulted in impaired OXPHOS and induced ferroptosis or apoptosis in all the AML cell lines analyzed. Genetic knockdown of CES1 confirmed the reduced cell survival observed with pharmacological inhibition. Furthermore, exogenous oleate supplementation partially rescued cell viability, highlighting a CES1-dependent lipid metabolic axis essential for cell survival under stress.

CONCLUSIONS

These data support the concept that targeting metabolic adaptation, specifically through CES1 inhibition, may represent a novel and effective strategy to impair leukemic cell survival in AML.

P233

ROLE OF KCASH2 DURING COLORECTAL INFLAMMATION AND TUMORIGENESIS

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BACKGROUND-AIM

The Hedgehog (Hh) signaling pathway plays a pivotal role in embryogenesis and tissue self-renewal and differentiation. Aberrations in Hh components or non-canonical activation of Hh signaling have been implicated in several tumors, and more recently in colorectal cancer (CRC), one of the most prevalent malignancies worldwide.
We previously identified KCASH2 as a tumor suppressor and Hh pathway inhibitor. Now we are investigating the impact of KCASH2 loss on CRC tumorigenesis both in vivo and in vitro.

METHODS

The effects of KCASH2 loss on Hh signaling during CRC tumorigenesis in vivo, where explored in KCASH2-knockout (KO) mice, following standard azoxymethane (AOM) and dextran sulfate sodium (DSS) protocol. Assessment of intestinal inflammation was conducted via immunohistochemistry. Modulation of the Hh pathway, along with inflammatory and apoptotic signaling cascades, was analyzed by Western blotting and qRT-PCR. Leukocyte populations were evaluated through flow cytometry. In vitro scratch assay was performed to assess epithelial repair.

RESULTS

Our data show that KCASH2 deficiency leads to activation of the main Hh transcription factor, GLI1. While KO mice do not develop spontaneous tumors, in silico analysis on human CRC databases revealed that KCASH2 is downregulated in IBD-associated CRC and inversely correlates with Gli1 expression.
KO mice following AOM/DSS, exhibit higher mortality, weight loss, colon shortening, and impaired recovery. Colons from KO mice showed altered type 2 immune responses—typically involved in tissue repair— enhanced NF-κB activity and reduced apoptosis. Coherently, in vitro data suggest that KCASH2-silenced cells exhibit defective wound healing after mechanical injury. Interestingly, KO mice exhibited larger and more numerous colon tumors compared to WT. Molecular analyses of tumor and adjacent tissue confirmed hyperactivation of the Hh signaling pathway.

CONCLUSIONS

Loss of KCASH2 promotes aberrant activation of the Hh pathway and contributes to CRC progression in the context of inflammation. Moreover, KCASH2 loss appears to affect type 2 immune responses and epithelial regenerative capacity. These results suggest that KCASH2 may represent a promising therapeutic target in inflammation-associated CRC.

P234

EPIGENETIC SIGNATURES AND ACCELERATED AGING IN PAINFUL DIABETIC NEUROPATHY: A COMPREHENSIVE DNA METHYLATION ANALYSIS

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BACKGROUND-AIM

Painful diabetic neuropathy (PDN) is one of the most disabling complications of type 2 diabetes, affecting up to 30% of patients and severely impairing quality of life. Although its pathophysiology remains unclear, recent epigenomic advances suggest that DNA methylation patterns not only regulate gene expression but may also estimate biological age, a marker of individual health status. However, the role of epigenetic alterations in PDN is still poorly defined. This study aimed to identify DNA methylation signatures associated with PDN and assess whether the condition is linked to accelerated epigenetic aging.

METHODS

We analyzed DNA methylation profiles using the Illumina EPIC array in two independent cohorts of diabetic patients with and without PDN (PLDN), recruited in Germany (72 PDN, 67 PLDN) and the UK (27 PDN, 65 PLDN). In the first cohort, high-resolution whole-genome DNA methylation analysis was assessed. In the second, we evaluated epigenetic aging using six categories of methylation-derived biomarkers: (1) standard epigenetic clocks, (2) PC-based enhanced clocks, (3) immune cell-type estimates, (4) biological trait predictions, (5) CRP-associated inflammation scores, and (6) plasma protein surrogates (EpiScores). Comparisons were made across patient groups and versus healthy controls.

RESULTS

Genome-wide analyses revealed distinct methylation signatures in PDN, including differentially methylated CpGs enriched in genes related to neuroinflammation, pain signaling, and neural development. Patients with PDN showed consistent signs of epigenetic age acceleration across multiple clocks. Immune deconvolution indicated reduced B lymphocytes and CD8+T cells and increased granulocytes in PDN. Furthermore, levels of several inferred plasma proteins, including GHR, MMP1, THBS2, and TGF-α, were significantly altered compared to PLDN.

CONCLUSIONS

Our findings provide the first robust evidence of unique methylation signatures and epigenetic aging acceleration in PDN. These changes may reflect underlying biological mechanisms contributing to neuropathic pain and offer potential biomarkers for early detection and risk assessment. Further validation in larger cohorts is required.

P235

MODULATION OF TNF-α-INDUCED INFLAMMATION AND OXIDATIVE STRESS IN HUVECS BY TWO PLANT-DERIVED EXTRACTS

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BACKGROUND-AIM

Inflammation and oxidative stress are widely recognized as major contributors to the development of "inflammaging"—a chronic, low-grade inflammatory state associated with aging. Reactive oxygen species (ROS) play a central role in the molecular processes driving cellular aging and tissue dysfunction. Numerous plant-derived compounds are known to modulate molecular signaling pathways, thereby attenuating both inflammation and oxidative stress. Human umbilical vein endothelial cells (HUVECs) represent a well-established in vitro model for studying endothelial responses to inflammatory stimuli and testing the efficacy of potential therapeutic agents. This study aimed to evaluate the effects of two different plant extracts, C43 and C210, on TNF-α-induced inflammation and oxidative stress in HUVECs, and to identify some of the underlying mechanisms of action.

METHODS

To establish the experimental conditions, cytotoxicity assays were first conducted to determine the safe concentration ranges and optimal exposure times for C43 and C210 in HUVECs at passages 4 and 7. Real-time RT-PCR and ELLA cytokine assays were employed to assess the anti-inflammatory and antioxidant responses following treatment with the extracts and TNF-α stimulation. Particular attention was given to identifying differences in cell viability and extract tolerance based on cell passage.

RESULTS

Both extracts showed defined safety windows in terms of concentration and exposure time in HUVECs, with passage 4 cells demonstrating greater viability and tolerance. Based on these findings, subsequent co-treatment experiments with TNF-α have been conducted under optimized conditions. Preliminary analyses indicate that both C43 and C210 may mitigate TNF-α-induced inflammatory and oxidative responses, as evidenced by modulated expression of cytokine markers and reduced ROS-related damage.

CONCLUSIONS

The plant extracts C43 and C210 exhibit promising anti-inflammatory and antioxidant properties in endothelial cells exposed to TNF-α, potentially through modulation of cytokine signaling and oxidative stress pathways. These findings support the potential use of specific phytoextracts in strategies aimed at counteracting endothelial dysfunction and inflammaging. Further investigations are warranted to fully elucidate the molecular mechanisms involved.

P236

TARGETING NADH HOMEOSTASIS THROUGH GPD2 INHIBITION AND METABOLIC STRESS INDUCES TUMOR REGRESSION IN COLORECTAL CANCER

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BACKGROUND-AIM

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.

METHODS

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.

RESULTS

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.

CONCLUSIONS

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.

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ABCAR: A NEW APPROACH COMBINING CAR-T CELL TECHNOLOGY WITH THE SECRETION OF SOLUBLE BLOCKING ANTIBODIES

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BACKGROUND-AIM

CAR-T cell therapy is immunotherapy in which T cells are genetically engineered to express a chimeric antigen receptor (CAR) that targets a specific protein on cancer cells. Once re-infused into the patient, these modified T cells can recognize and kill cancer cells more effectively. The therapy has shown remarkable success in treating blood cancers such as B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma. However, the technology has yet to impact the treatment of solid tumours. In this work, we optimised the choice of antigen as well as the epitope for the chimeric fusion and innovated the technology by combining it with secreting antibodies from the same CAR-T cell. We call this ABCAR.

METHODS

Jurkat cells, a human T-lymphocyte cell line, were lentiviral transduced with DNA constructs encoding a CAR targeting MICA or B7H6 combined with an in-frame coding sequence for secreted scFv anti-TGFbeta, separated by the P2A ribosomal skip sequence. Transduced cells were selected by FACS for the presence of the CAR and cultured in the presence of puromycin. CAR-positive Jurkat cells were co-cultured with HepG2 target and CHO control cells. Activation was measured by flow cytometry using anti-CD25, -CD69 and -CD3z to detect effector cell activation as well as the presence of TNFa in the culture medium measured by ELISA. Blocking by anti-TGFb scFv was demonstrated with a reporter assay.

RESULTS

A two-plasmid, mouse- and human-specific lentiviral ABCAR targeting system was generated. Jurkat cells were successfully transduced, sorted, and showed CAR-mediated activation on cells expressing the target antigen (HepG2) which was absent in co-culture with CHO control cells. ABCAR mediated upregulation of Bax, an apoptotic marker, and loss of viability in HepG2 cells was demonstrated. Finally, recombinantly-produced anti-TGFb scFv showed dose-dependent blocking of the TGFb receptor activation in a cell-based reporter system.

CONCLUSIONS

We designed a strategy to conveniently place newly developed scFv antibody fragments into the CAR or secreted position (AB) within a plasmid backbone for lentiviral transduction. We successfully targeted two liver cancer antigens and showed activation of the transduced Jurkat cells and killing of the effector cells. IgG-derived anti-TGFb scFv was able to block ligand-induced receptor activation offering secondary functionality to CAR-T cells, introducing the "ABCAR" concept.

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FOLIC ACID-FUNCTIONALIZED MESOPOROUS SILICA NANOPARTICLES AS A TARGETED DELIVERY SYSTEM FOR MEBENDAZOLE IN FOLATE RECEPTOR-POSITIVE CANCER CELLS

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BACKGROUND-AIM

Breast cancer is the most frequently diagnosed cancer among women worldwide, accounting for ~23.8% of all new oncological cases. In recent years, mebendazole (MBZ), an orally available FDA-approved anthelmintic drug, has emerged as a promising candidate for drug repurposing in oncology, showing anticancer activity across various tumors, including breast cancer. To overcome MBZ's low oral bioavailability and improve drug accumulation in tumor tissue, we developed a folate-functionalized mesoporous silica nanoparticle system (FOL-MSN-MBZ) for the targeted delivery of MBZ to folate receptor-positive (FR+) breast cancer cells.

METHODS

FOL-MSN-MBZ was synthesized by covalently grafting folic acid (FOL) onto MSNs' external surface. MBZ was then loaded into the nanopores via a two-step impregnation procedure, achieving a drug content of 8.80%. Free MBZ cytotoxicity was evaluated using the MTT assay and 3D spheroid models. Cell lines were selected based on folate receptor (FR) expression, assessed by Western blot. Subsequently, we investigated the selective therapeutic efficacy of FOL-MSN-MBZ in FR-positive (FR+) breast cancer cell lines—including parental MCF-7, their tamoxifen-resistant counterpart (TamR), MDA-MB-231, and MDA-MB-468—as well as its effects on FR-negative (FR-) non-cancerous 3T3-L1 fibroblasts, using cell proliferation assays.

RESULTS

Our data demonstrate that MBZ causes death in both 2D and 3D MCF-7 and TamR cell cultures in a dose-dependent manner. FOL-MSN-MBZ effectively induces selective cell death in all the FR+ breast cancer cell lines, while showing minimal toxicity toward FR- normal 3T3-L1 fibroblasts. In contrast, free MBZ demonstrated cytotoxic effects across all tested cell lines, including the non-cancerous ones.

CONCLUSIONS

Our results indicate that FOL-MSN-MBZ enables targeted delivery of MBZ, inducing cytotoxicity specifically in FR+ tumor cells, with minimal impact on normal cells. Ongoing studies aim to evaluate if this nanodevice can reproduce the antitumoral effects of the free drug -such as cell cycle arrest, microtubule destabilization, and apoptosis induction- with enhanced specificity for tumor cells.

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CD16+ CD8+ T CELLS EMERGING FOLLOWING MRNA VACCINATION FOR COVID-19 CAN INFILTRATE HUMAN COLORECTAL CANCER

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BACKGROUND-AIM

Interesting reports revealed the potential of mRNA-based COVID-19 vaccines to stimulate anticancer immunity and promote tumor regression. Two doses of mRNA COVID-19 vaccine induce strong inflammatory responses with enhanced CD8+ T cell subset infiltration into the tumor microenvironment. Looking at the specific subsets of CD8+ T cells, a significant increase in cytolytic CD8+ expressing TIGIT was observed in tumours post vaccination. We have recently reported that mRNA vaccination expands a subset of unconventional CD8+ T cells bearing CD16 and endowed with innate cytotoxic potential and ADCC capability. In this study aimed to determine whether CD16+CD8+ T cells accumulate in colorectal cancer (CRC) and may account for the increase of intratumoral TIGIT+CD8+T cells observed upon vaccination.

METHODS

Peripheral blood from 20 healthy donors and tissue specimens from 10 CRC patients receiving BNT162b2 mRNA vaccine were collected to analyze T-cell compartment by using multiparametric flow cytometry. CRC tissues were further examined using IHC and IF staining.

RESULTS

We observed that CD16+CD8+ cells significantly upregulate TIGIT expression following mRNA vaccination, while it decreases in conventional CD8+ T cells. TIGIT+CD16+CD8+ T cells display high levels of activating receptors and cytolytic molecules within CD8 T cell compartment and show a superior killing effect against cancer cells. Notably, TIGIT+CD16+ T cells accumulate in tumor tissues of CRC patients, who previously received mRNA COVID-19 vaccine, and are reactive to SARS-CoV-2 peptides, suggesting their derivation from vaccine-induced T cell response. They also express ICOS, a costimulatory molecule involved in B cell activation and survival and, accordingly, a fraction of CD8+ T cells infiltrating CRC co-localize with B cells and IgG-bearing tumor cells, which may therefore be targets for ADCC.

CONCLUSIONS

Taken together, these observations raise the hypothesis that mRNA COVID-19 vaccination might enhance antitumor immunity by promoting the expansion of CD16+CD8+T cells capable of infiltrating human tumors and potentially mediating cytotoxic responses, including ADCC, against IgG-coated cancer cells.

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MULTIPLE MYELOMA RESISTANCE TO NK CELL KILLING IS PRIMARILY MEDIATED BY NON-CLASSICAL HLA-E MOLECULE

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BACKGROUND-AIM

Natural killer (NK) cells are critical for immune surveillance of multiple myeloma (MM), a plasma cell malignancy with limited curative options. However, MM cells can evade NK cell cytotoxicity by retaining expression of HLA class I molecules, including the non-classical HLA-E, suggesting that inhibitory signaling through receptors like killer cell immunoglobulin-like receptors (KIRs) and CD94/NKG2A may play a critical role in regulating NK cell response in MM.

METHODS

We investigated the impact of inhibitory signaling on NK cell function using a multidimensional approach combining high-dimensional flow cytometry and single-cell technologies.

RESULTS

Our data revealed that HLA-E is highly expressed on malignant plasma cells and increases with disease progression, whereas HLA-I expression slightly decreases. Accordingly, functional assay demonstrated that HLA-E exerts a stronger inhibitory effect on NK cell cytotoxicity than HLA-I molecules. This was further supported by CRISPR/Cas9 screening, which identified HLA-E as the top gene conferring resistance to NK lysis. Moreover, engagement of NKG2A by HLA-E reprograms NK cells toward a less cytotoxic phenotype, characterized by downregulation of CD16, PRF1, and GZMB. Single-cell RNA-seq analysis of NK cells from MM patients corroborated these findings, demonstrating progressive loss of effector gene expression during disease evolution. On the other hand, stimulation via the activating receptor NKG2D downregulated NKG2A expression, thereby counteracting HLA-E-mediated inhibitory signal.

CONCLUSIONS

Our study identifies HLA-E as a dominant driver of NK cell dysfunction in MM, suggesting that targeting HLA-E/NKG2A axis may restore NK cell anti-myeloma response. Additionally, targeting NKG2D provides a complementary approach to overcome HLA-E–mediated inhibition and further enhance NK cell function.

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GM-CSF–PRODUCING RORGT ILC3S ACCUMULATED IN HUMAN COLORECTAL CANCER AND CORRELATED WITH TERTIARY LYMPHOID STRUCTURE-ASSOCIATED CELL TYPES

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BACKGROUND-AIM

Accumulating evidence indicates that group 3 innate lymphoid cells (ILC3) play a crucial role in shaping gut tumor immunity. These cells contribute to the modulation of the tumor microenvironment through the production of cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF). However, the specific mechanisms by which ILC3-derived GM-CSF influences colorectal cancer (CRC) immunity remain poorly understood.

METHODS

Tissue samples from 30 patients with CRC were collected to investigate the contribution of ILC3 in modulating the antitumor immune response using multiparametric flow cytometry and single-cell transcriptomic analysis.

RESULTS

RNA-seq and ex vivo analyses revealed that ILC3s are the predominant source of GM-CSF in colorectal cancer (CRC) patients, both in peripheral blood and within tumor tissues. In addition to GM-CSF production, CRC-associated ILC3s acquire lymphoid tissue–like characteristics and localize within tertiary lymphoid structures (TLSs) in both human and murine CRC, where they co-localize with T cells suggesting potential crosstalk between these cell types. In this regard, stimulation of primary tumor-samples with recombinant GM-CSF activates T cell compartment, promoting a T follicular helper (Tfh)-like phenotype marked by increased PD-1 expression and CXCL13 production. Accordingly, anti-PD1 synergizes with GM-CSF to enhance these effects. Consistent with these findings, GM-CSF–positive CRCs display elevated expression of TLS-associated genes compared to GM-CSF–negative tumors.

CONCLUSIONS

Together, these results highlight the importance of GM-CSF as a critical cytokine through which ILC3s shape the tumor immune microenvironment in the context of CRC. Moreover, we uncover a previously unappreciated aspect of GM-CSF–mediated immune regulation, suggesting a potential role in supporting the organization and function of tertiary lymphoid structures within the tumor microenvironment.

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DIFFERENTIAL EXPRESSION OF MRNA 3'-END KRAS IN HUMAN CANCER

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BACKGROUND-AIM

Many studies found that cancer cells favor shorter 3'UTRs in oncogenes and proliferation genes The lengthening events also occur with a variable frequency in human cancers. KRAS is a prognostic marker in many cancers , and its exceptionally long 3'UTR end possesses many cis- and trans-acting factors that act as genetic biomarkers predictive of prognosis, diagnosis, and treatment of many cancers. Here, we tested differential 3'UTR KRAS lengths expression in human cancers using an independent cohort of primary samples. We measured differential isoform abundance, and its impact on encoded protein levels.

METHODS

PCR-based data on relative expression of long and short 3'untranslated region of KRAS were obtained by designing three specific primers (short 3'-UTR; middle 3'-UTR and distal 3'-UTR). RT-qPCR analysis measured ##ct KRAS in an independent cohort of human primary samples (patient tumor specimens as FFPE or low-passage cultures) encompassing glioblastoma, lung adenocarcinoma and squamous carcinoma, infiltrating breast carcinoma and colon carcinoma. Moreover, semiquantitative western-blot analysis was performed to measure the impact on encoded protein levels.

RESULTS

We found that 3'-UTR KRAS transcripts are expressed in a cell-type-specific manner. Particularly, glioblastoma primary cultures show an extensive regulation of 3'UTR lenghts; we quantifyed the mRNA fold-induction of primary and long-term cultures over normal counterpart (NHA) and distinct glioblastoma subtype (stem-like versus non-stem like). The exceptionally long 3'UTR KRAS transcript was undetectable in almost all the samples, with a few exceptions (i.e. cultured mammary epithelial cell lines MCF-10). Human breast cancers showed a remarkable 3' UTR KRAS shortening in FFP tissues compared to commercially cell lines.

CONCLUSIONS

In contrast to its mutational status the transcriptional landscape of the KRAS gene remains largely unknown. Recently the identification of multiple transcript KRAS isoforms (39 novel KRAS mRNA transcript variants) whose the functional relevance of the presence of multiple transcript isoforms is largely unexplored. Further analysis will determine 3'UTR KRAS length changes in cancer as a genetic biomarker or in RNA-based anti-cancer therapeutics.

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SARCOPENIC OBESITY AND PHYSICAL FRAILTY: INSIGHTS FROM A STUDY OF OLDER ITALIANS AND CENTENARIANS

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BACKGROUND-AIM

Aging is accompanied by significant changes in body composition (BC), including increased fat mass, reduced lean mass, and decreased bone mineral density. These alterations adversely affect metabolic health, physical performance, and overall functionality, and are associated with conditions such as obesity, type 2 diabetes, sarcopenia, and frailty. While the contribution of sarcopenia to frailty is well established, the role of adiposity remains less defined. This study aims to investigate the relationship between BC parameters and frailty status in older adults, with particular attention to sarcopenic obesity and its implications for healthy aging.

METHODS

A cross-sectional analysis was conducted on 295 community-dwelling Italian individuals aged 65–79 years, stratified by frailty status, in addition to a comparison group of centenarians. Body composition was assessed using dual-energy X-ray absorptiometry (DXA), including measures of fat mass, lean mass, and bone mineral density. Inflammatory markers and nutritional status indicators were also evaluated to explore their associations with BC and frailty.

RESULTS

Centenarians demonstrated BC profiles comparable to robust individuals aged 65–79, with the exception of bone-related parameters. In contrast, frail participants exhibited significantly higher fat mass and reduced lean mass compared to both non-frail subjects and centenarians ($p < 0.05$). These findings suggest a strong association between frailty and sarcopenic obesity. Moreover, inflammatory and nutritional markers further differentiated the frail group, supporting the relevance of these physiological domains in the aging process.

CONCLUSIONS

The physical frailty phenotype is closely linked to sarcopenic obesity, highlighting the detrimental role of unbalanced body composition in aging. Centenarians' preserved BC features, despite their advanced age, suggest that maintaining lean mass and limiting excess adiposity may contribute to longevity and functional independence. These findings underscore the importance of targeted interventions promoting muscle preservation and fat control to support healthy aging.

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