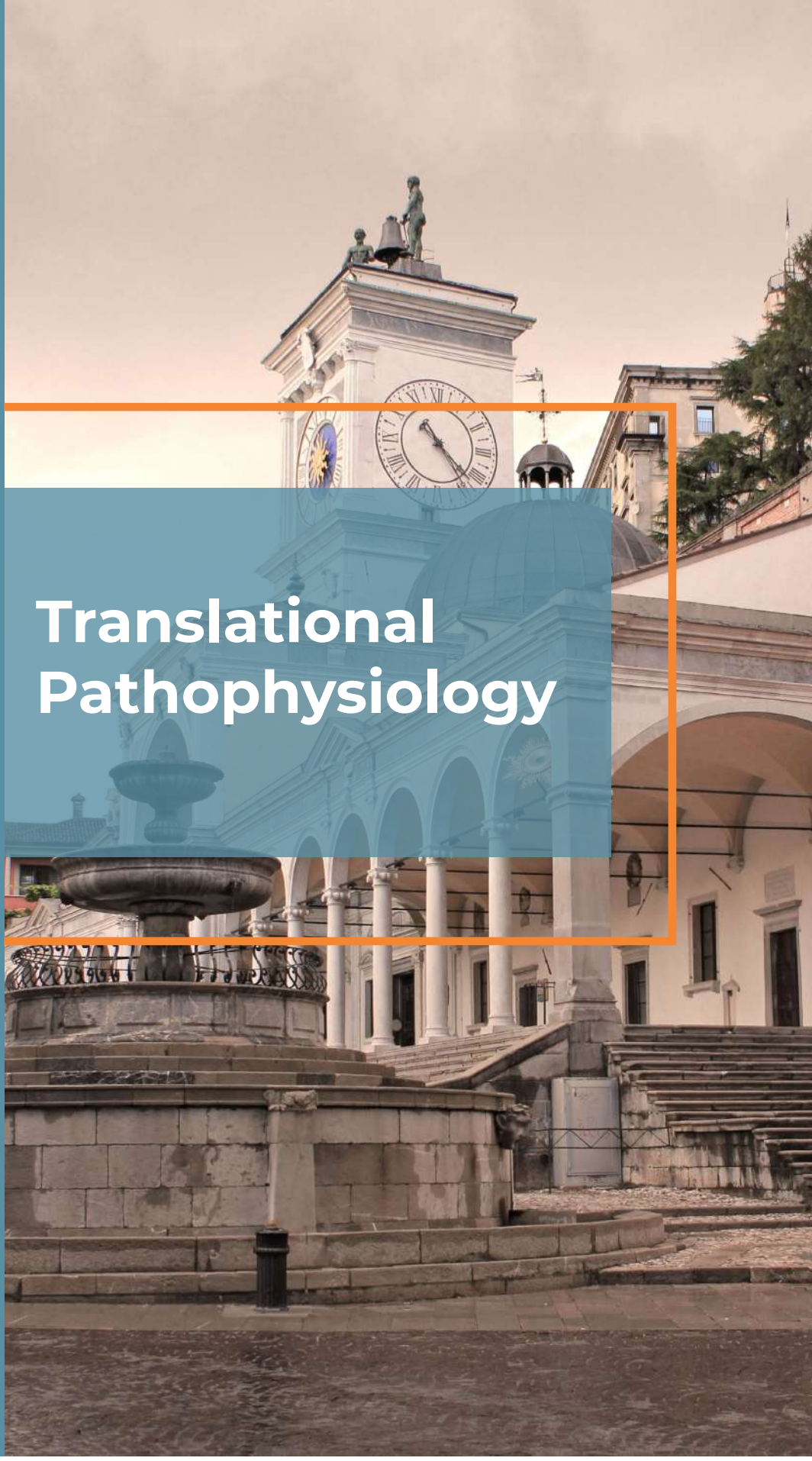

Translational Pathophysiology

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ABSTRACT BOOK

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SIP MET

P001

SARCOPENIA AND INFLAMMAGING; THE EFFECTS OF FUNCTIONAL FOODS FOR PROMOTING HEALTHY AGING

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BACKGROUND-AIM

Sarcopenia is a disorder characterized by a generalized reduction in muscle mass and strength and related to important negative clinical outcomes, with a series of economic and social impli-cations, including impaired mobility, loss of quality of life, hospitalization and death. Inflammag-ing is a state of low-grade systemic inflammation which plays an important role in age-related diseases as sarcopenia. Sarcopenia is also linked to chronic diseases such as obesity, diabetes and metabolic syndrome and inflammatory signals, coming from these diseases, influence negatively skeletal muscle.
Therefore, sarcopenia markers, are urgently needed especially related to musculoskeletal, hor-monal and biochemical status. Several papers, agreed on evaluating anthropometric indices, hormonal markers including cortisol, inflammatory markers including C-reactive protein (CRP) and interleukin-6 (IL-6), and vitamin D concentration, which deficiency of which correlates with increased inflammation and sarcopenia

METHODS

We have conducted a blindly, against placebo, nutritional intervention for four weeks on a court of 48 volunteer subjects (aged spanned between 60 to 75 years old) divided into two experi-mental groups. Control lettuce group and lettuce group biostimulated with Ecklonia species brown seaweed. The subjects consumed 100 grams of lettuce with or without biostimulation with Ecklonia species brown seaweed for 4 weeks. Blood samples and anthropometric and nutritional analysis were conducted at the beginning of recruitment and after 4 weeks.

RESULTS

The results showed changes in biochemical and anthropometric markers of sarcopenia. Subjects who consumed for four weeks biostimulated with Ecklonia species brown seaweed lettuce showed a reduction in levels of cortisol, inflammatory biomarkers such as c-reactive protein (CRP) and IL-6 and vitamin D.

CONCLUSIONS

These preliminary data suggest that brown seaweed supplementation enriched functional foods may be a functional choice that can be proposed to improve sarcopenia associated with aging and perhaps age associated mineral deficency.

P002

HIGH RISK ATHEROSCLEROTIC PATIENTS SHOW AN ENHANCED ADAPTIVE RECONFIGURATION OF NATURAL KILLER CELLS IN RESPONSE TO CYTOMEGALOVIRUS

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BACKGROUND-AIM

Human cytomegalovirus (HCMV) is associated with atherosclerosis and destabilization of atherosclerotic plaques. In some patients, HCMV promotes a marked reconfiguration of the natural killer (NK) cell compartment characterized by a distinct phenotype and antibody-dependent enhanced functional capabilities, including cytokine production. IFNγ producing NK cells have been implicated in atherosclerosis progression, apparently in association with cytomegalovirus infection. Yet, the precise role of NK cells in atherosclerotic plaque destabilization and the molecular mechanisms underlying HCMV-associated atherosclerosis progression remain open issues. This study aims to investigate the potential impact of the HCMV-induced reconfiguration of the NK cell compartment in the pathogenic mechanisms underlying atherosclerotic plaque instability.

METHODS

A total of 64 patients were enrolled in a follow-up protocol for carotid artery stenosis. Patients were classified following conventional criteria as bearing high-risk plaques (High-risk patients- HR patients) or low-risk plaques (Low-risk patients- LR patients). High-risk patients underwent carotid endarterectomy according to the European Society for Vascular Surgery (ESVS) guidelines. Carotid plaques and preoperative blood samples were obtained from all patients to be processed.
Multiparametric flow cytometry was used to evaluate NK cell phenotype and functionality.

RESULTS

Adaptive- non-conventional NK cells are enriched in high-risk atherosclerotic HCMV seropositive patients and display an increased expression of NKG2D.
Remarkably, we observed that FCεR1γ- non-conventional NK cells increase upon plaque destabilization in peripheral blood and accumulate in carotid plaques of high-risk atherosclerotic patients by upregulating markers for tissue residency.
Moreover, NK cells from High-Risk HCMV seropositive patients have enhanced antibody-dependent effector functions compared to Low-Risk patients and antibody-dependent IFN-

CONCLUSIONS

In conclusion, we provide new data involving memory-like NK cells and NKG2D in atherosclerotic plaque destabilization, further suggesting that their analysis may provide useful indications to identify high-risk patients that might benefit from early surgical intervention and/or closer follow-up.

P003

IDENTIFICATION OF SMURF PROTEINS AS NOVEL DIRECT REGULATORS OF THE ONCOGENIC HEDGEHOG/GLI1 SIGNALING PATHWAY

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BACKGROUND-AIM

GLI1 is a transcriptional factor known as the main effector of the Hedgehog (Hh) pathway, which is involved in embryonic development, staminality and cell proliferation. Dysregulated GLI1 transcriptional activity is strongly associated with tumorigenesis, as in the case of the Medulloblastoma (MB). Since GLI1 can be activated by both canonical Hh- mediated signals and non-canonical signals mediated by other oncogenic pathways, it appears to be a most promising therapeutic target. For these reasons, a deeper understanding of the mechanisms modulating Gli1 stability and activity has become crucial. Among these mechanisms, protein ubiquitination and degradation have been shown as relevant in GLI1 protein turnover. We characterize here the role of the two HECT-E3 Ubiquitin Ligases SMURF1 and SMURF2, in the modulation of Hh/GLI1 signaling.

METHODS

The effects of SMURFs on GLI1 and Hh pathway have been investigated in HEK293T cells, MB-DAOY and MB-D283. Cells transfection has been performed using liposomes-based reagents. Hh pathway modulation has been monitored by RT-qPCR and luciferase reporter assays. Protein levels have been analyzed by Western blot (WB). Interaction between GLI1 and SMURF proteins has been demonstrated by Co-Immunoprecipitation (Co-IP) in HEK.293T cells, by in situ proximity ligation assay and in vitro by using the cell-free system of rabbit reticulocyte lysates. GLI1 ubiquitin levels has been investigated by ubiquitination assays, both in vitro and in HEK-293T cells.

RESULTS

We demonstrated that SMURFs overexpression leads to a reduction of exogenous GLI1 protein levels and, accordingly, it promotes a downregulation of Hh-GLI1 transcriptional activity. In order to clarify the mechanism used by SMURF proteins to induce GLI1 degradation, we performed Co-IP and in vitro-binding assays which confirmed a direct interaction between SMURFs and GLI1. Moreover, SMURFs overexpression increases GLI1 ubiquitination levels both in vitro and in cells, and this modification leads to GLI1 degradation by proteasome. Finally, we observed that SMURFs overexpression in medulloblastoma cell lines is able to reduce their proliferation rate by inducing GLI1 protein degradation.

CONCLUSIONS

Taken together, our evidence indicates a previously unknown role for SMURF proteins on GLI1 post-translational regulation, suggesting new approaches and therapeutic strategies in the treatments of tumors exhibiting dysregulation of GLI1 activity.

P004

HEALTH TECHNOLOGY ASSESSMENT FOR THE INTRODUCTION OF ABBOTT I-STAT TBI PLASMA TECHNOLOGY IN THE EMERGENCY DEPARTMENTS FOR THE RISK STRATIFICATION OF THE MILD TRAUMATIC BRAIN INJURY.

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BACKGROUND-AIM

Each year in Italy, approximately 670,000 traumatic brain injuries (TBIs) are classified as mild (mTBI), significantly impacting healthcare facilities. Currently, the gold-standard method for diagnosing traumatic brain injury is computed tomography (CT). Following various research studies conducted in the USA and considering the challenges related to emergency department admissions, a multidisciplinary research group conducted a Health Technology Assessment (HTA) regarding the potential use of the Abbott i-STAT™ TBI Plasma Test in the diagnostic pathway for mTBI in adults. This study focuses on the quantification of two brain injury biomarkers in peripheral blood (UCH-L1 and GFAP) and analyzes the benefits in terms of reducing the number of CT scans and the length of stay in the emergency department.

METHODS

1. **Definition of PICO model and search strategy:** Selection of relevant scientific studies from the literature and identification of efficacy data.
2. **Questionnaire administration:** A survey was conducted with a panel of 11 healthcare professionals from various fields and institutions.
3. **Use of the EUnetHTA Core Model:** Selection of 9 dimensions, prioritized based on the responses received.
4. **Construction of radar charts:** Comparison of the standard pathway with the innovative one.
5. **Multi-Criteria Decision Analysis (MCDA):** Direct analysis between the two pathways.

RESULTS

In 2023, at the ASST of Lodi, 1500 emergency department admissions were analyzed to compare the traditional and innovative pathways from an economic and financial perspective, with the innovative pathway reparametrized based on literature data. Following the expert interviews, certain dimensions were prioritized more due to their clinical perspective. MCDA results highlighted a higher score for the new pathway, with a minimal difference despite a 40% reduction in CT scans and emergency department stay time.

CONCLUSIONS

1. **Interview additional experts:** Include technical-administrative experts not adequately considered in the initial assessment to obtain a broader prioritization of dimensions beyond the clinical perspective.
2. **Multicenter study:** Validate the efficacy data present in the literature through a multicenter study.
3. **Development of a PDTA:** Establish a diagnostic and therapeutic pathway based on Point of Care Testing (POCT) devices.

P005

NOVEL MOLECULAR MECHANISMS TRIGGERING BREAST CANCER RESISTANCE TO THE CDK4/6 INHIBITOR PALBOCICLIB

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BACKGROUND-AIM

The cyclin D1-cyclin dependent kinases (CDK)4/6 inhibitor palbociclib, used in combination with endocrine therapy, has demonstrated therapeutic efficacy in estrogen receptor (ER)-positive and HER2-negative breast cancer patients. However, the resistance to palbociclib underscores the need to identify new targets for more comprehensive treatments of these patients.

METHODS

Breast cancer cells resistant to palbociclib and cancer-associated fibroblasts (CAFs) derived from mammary tumors were generated and used as a model system. Gene silencing and overexpression experiments, real-time PCR, immunoblotting, chromatin immunoprecipitation assays as well as cell viability, colony, and 3D spheroid formation assays allowed us to investigate whether the G protein-coupled estrogen receptor (GPER) may play a role in the functional interactions among components of the tumor microenvironment, leading to palbociclib resistance. Bioinformatics analyses and k-means clustering of clinical and expression data from large cohorts of breast cancer patients were performed to evaluate the clinical significance of novel mediators involved in the resistance to palbociclib.

RESULTS

We ascertained that a down-regulation of ERα levels along with an increased expression of GPER occur in palbociclib-resistant breast cancer cells. Moreover, we assessed that the epidermal growth factor receptor (EGFR) is up-regulated in these cells and interacts with the GPER promoter region, thereby enhancing the expression of GPER, toward BC cells resistance to palbociclib treatment. Next, we found that palbociclib induces pro-inflammatory transcriptional changes through GPER-dependent signaling in CAFs. Of note, by performing co-culture assays we demonstrated that GPER facilitates a functional liaison between breast cancer cells and CAFs, then contributing to a reduced palbociclib sensitivity.

CONCLUSIONS

Our findings provide new insights into the involvement of GPER in the resistance to palbociclib in breast cancer cells. Therefore, therapeutic strategies targeting GPER might be explored in order to prevent and/or overcome palbociclib resistance in breast cancer patients.

P006

TWO NOVEL CANCER-ASSOCIATED FIBROBLASTS (CAFS) GENE SIGNATURES DISPLAY PROGNOSTIC VALUES IN BREAST AND PROSTATE CANCER PATIENTS

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BACKGROUND-AIM

Tumor-derived gene signatures have been developed in order to improve the outcome prediction and the therapeutic strategy for the two most commonly diagnosed cancers worldwide, namely breast and prostate tumors. Molecular signatures obtained from important components of the tumor microenvironment, such as cancer-associated fibroblasts (CAFs), have been studied. However, the prognostic value of CAFs-derived signatures in breast and prostate cancers remains to be fully disclosed.

METHODS

The whole transcriptome profile of CAFs isolated from breast and prostate cancer patients was identified by RNA sequencing technology. The differentially expressed genes (DEGs) that characterize breast and prostate CAFs were intersected with data available from public datasets based on RNA-seq profiles of patients affected by breast and prostate tumors. The biological significance of the DEGs was explored by pathway enrichment analyses, whereas K-means clustering was applied to construct CAFs-related gene signatures specific for breast and prostate cancer as well as to stratify high and low gene expression clusters of independent cohorts of patients. Differences in the outcome of the patients were predicted by the Kaplan-Meier curves and log-rank tests. The validation of the clustering results was achieved by decision-tree analysis followed by boosting calculations to strengthen the results obtained.

RESULTS

We first identified a breast CAFs signature including 8 genes (ITGA11, THBS1, FN1, EMP1, ITGA2, FYN, SPP1, EMP2) belonging to the “Focal adhesion” pathway. Of note, poor prognostic values were displayed by breast cancer patients clustered on the basis of the high expression of these genes. Next, we established a prostate CAFs-related signature counting 11 genes (IL13RA2, GDF7, IL33, CXCL1, TNFRSF19, CXCL6, LIFR, CXCL5, IL7, TSLP, TNFSF15) that are predictive of a poor prognosis when expressed at low levels in prostate cancer patients. Supervised classification techniques allowed the validation of both gene signatures with high accuracy (≥ 90%) in multiple independent RNA-seq cohorts.

CONCLUSIONS

We developed two novel CAFs-related gene signatures that might serve as prognostic indicators and novel biomarkers for a more effective management of patients with breast and prostate cancer.

P007

ASSESSMENT OF A GENE SIGNATURE DERIVED FROM CANCER-ASSOCIATED FIBROBLASTS (CAFS) OF MALE BREAST TUMOR PATIENTS

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BACKGROUND-AIM

Male breast cancer (BC) is an aggressive tumor accounting for approximately 1% of all cancers in men and 1% of BC cases globally. The tumor microenvironment is a complex and evolving entity that promotes cancer progression. A thorough identification of the microenvironment surrounding the male BC could offer crucial insights for setting innovative therapeutic approaches. In this context, the isolation of cancer-associated fibroblasts (CAFs) from male BC has not been accomplished yet, thus representing a unique opportunity for a better comprehension of this rare tumor.

METHODS

RNA-sequencing analysis was performed to identify the differentially expressed genes (DEGs) between male and female breast CAFs. The ReactomePA and clusterProfiler packages served for pathway and gene ontology (GO) enrichment analyses, respectively. Considering that no datasets from male BC patients are available, the k-means algorithm was used to build a CAFs-derived gene signature to cluster patients of the TCGA dataset affected by the unique male cancer such as prostate tumor. Survival analyses were performed employing Kaplan-Meier curves and log-rank tests. The clustering results were validated through the decision-tree classification technique. All analyses were performed in R Studio.

RESULTS

RNA-sequencing studies revealed 775 DEGs in CAFs isolated from male BC compared to those derived from female BC. Bioinformatic analyses indicated that the genes highly expressed in male breast CAFs are involved in tumor invasiveness and metastasis. Specifically, the analysis identified 12 genes that characterize the expression profile of male breast CAFs and associate with invasive processes. Given the lack of datasets containing clinical and transcriptional data of male BCs, additional analyses using machine learning techniques were performed on data derived from patients with prostate cancer. Our results identified a molecular signature for prostate cancer consisting of 9 (ASPN, COL4A1, COL4A2, COL5A3, COMP, FN1) out of the 12 genes that characterize the expression profile of male breast CAFs. The aforementioned 9 genes were shown to predict the aggressiveness of prostate malignancy with a power of 94%, thus providing important biomarkers for prognostic information and therapeutic strategies.

CONCLUSIONS

We assessed a gene signature derived from male breast CAFs even showing the potential to predict prostate cancer prognosis and to serve as a valuable indicator for tailored therapeutic strategies.

P008

ROLE OF HSF1 IN CHOLANGIOCARCINOMA

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BACKGROUND-AIM

Intrahepatic cholangiocarcinoma (iCCA) is a lethal primary liver tumor characterized by clinical aggressiveness, poor prognosis, and scarce therapeutic possibilities. Therefore, new treatments are urgently needed to render this disease curable. Since cumulating evidence supports the oncogenic properties of the Heat Shock Factor 1 (HSF1) transcription factor in various cancer types, we investigated its pathogenetic and therapeutic role in iCCA.

METHODS

Levels of HSF1 were evaluated in a vast collection of iCCA specimens. The effects of HSF1 inactivation on iCCA development in vivo were investigated using three established oncogene-driven iCCA mouse models. In addition, the impact of HSF1 suppression on tumor cells and tumor stroma was assessed in iCCA cell lines, cancer-associated fibroblasts (CAFs), and patient-derived organoids.

RESULTS

Human preinvasive, invasive, and metastatic iCCAs displayed widespread HSF1 upregulation, which was associated with a dismal prognosis of the patients. In addition, hydrodynamic injection of a dominant-negative form of HSF1 (HSF1dn), which suppresses HSF1 activity, significantly delayed cholangiocarcinogenesis in AKT/NICD, AKT/YAP, and AKT/TAZ mice. In iCCA cell lines, CAFs, and patient-derived organoids, administration of the HSF1 inhibitor KRIBB11 significantly reduced proliferation and induced apoptosis. Cell death was further augmented by concomitant administration of the Bcl-2/Bcl-xL inhibitor navitoclax. Furthermore, tumor cells deprived of HSF1 displayed lower proliferation capabilities, increased apoptosis, and reduced glycolysis.

CONCLUSIONS

The present data underscore the critical pathogenetic and prognostic role of HSF1 in cholangiocarcinogenesis.

P009

CARDIAC COL1A2 AS A POTENTIAL EARLIER HUB GENE IN OBESITY-INDUCED MALADAPTIVE HEART REMODELING IN DIO MICE MODEL

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BACKGROUND-AIM

Obesity cardiomyopathy refers to the structural, functional, and metabolic abnormalities of the heart caused by obesity. Indeed, if it left untreated can cause ventricular hypertrophy, diastolic dysfunction, and cardiac fibrosis in adulthood. The non-cardiac cells (non-CCs) play an important role in obesity-induced extracellular matrix (ECM) responses. ECM in normal heart is usually composed of type I collagen and controlled by Col1a2 gene. Under obesogenic stimuli, ECM reorganization driven by non-CCs leads to increased transforming growth factor-β (Tgf-β) activity, and cardiomyopathy with loss of cardiac function over time. Little is currently known about pro-fibrotic ECM reorganization and remodeling occurring in the early stage of adult age, between the second and third decade. Here, we investigated the role of Col1a2 expression gene from the heart of diet-induced obesity (DIO) mice, and their association with cardiac ECM rearrangement in the early stage of adulthood.

METHODS

12 six-week-old male C57BL/6N DIO-mice (Charles River Laboratories) divided into two groups and fed for 20 weeks as follows: At the age of 26 weeks. The mice were sacrificed through exposure to atmosphere saturation of carbon dioxide for 15 min. Heart were collected, subdivided in two parts: the first one for genomic analysis and total collagen quantification 2) the second one for Masson's Trichrome staining. The Italian Ministry of Health approved all animal procedures (Number 5AD83.N.G1Q).

RESULTS

Genomic results showed that in HF heart, cardiac tissue starts to modify toward a pro-fibrotic ECM rearrangement than CTR. HF diet in DIO-mice aged matched to 20-30 years old human, promotes ECM deposition, through Col1a2 gene up-regulation which positive correlates with Acta2 and Tgfb1 family genes, the main actors of tissue remodeling. Sircol analysis showed that HF cardiac biopsies presented higher collagen content than CTR heart, although Masson's trichrome staining showed no difference in HF than CTR group.

CONCLUSIONS

Our data highlight the role of Col1a2 in pro-fibrotic ECM rearrangement in HF DIO-heart with 6 months of age, suggesting that Col1a2 gene can be considered a hub gene of obesity induced ECM remodeling at the beginning of adulthood.

P010

CO-STIMULATORY SIGNALS MEDIATED BY ICOS INFLUENCE THE HEPATIC EXPANSION OF AUTO-AGGRESSIVE CD8+ T-CELLS IN NASH

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BACKGROUND-AIM

Recent evidence indicated that cytotoxic $CD8^+$ T-lymphocytes play an important role in the progression of non-alcoholic steatohepatitis (NASH) toward fibrosis. The Inducible T-cell Co-Stimulator (ICOS) present on T lymphocytes and its ligand ICOSL (B7h) expressed on myeloid cells are members of the B7/CD28 family and play multiple roles in immunity by regulating T-cell activation/survival. From the observation that ICOS-expressing $CD8^+$ T-cells are important in driving liver healing following acute injury, we have here investigated the possible involvement of ICOS-ICOSL dyad in modulating T-cell functions during NASH evolution.

METHODS

ICOS and ICOSL were investigated in experimental models of NASH based on mice feeding with choline/methionine deficient (MCD) or a cholesterol-enriched Western (WD) diets

RESULTS

Liver CD8+ T cells expressing ICOS expanded in animal models of NASH in parallel with an up-regulation of ICOSL in $CD11b^{high}/F4\text{-}80^+$ monocytes/macrophages (MoMF). Mice deficient for ICOS receiving the MCD diet for 6 weeks had milder steatohepatitis. This effect was confirmed in mice fed with the WD diet for 24 weeks, which also showed reduced hepatic fibrosis. The characterization of $ICOS^+/CD8^+$ T-cells in WD-fed mice showed that they featured C-X-C Motif Chemokine Receptor 6 (CXCR6) and Programmed cell Death Protein-1 (PD-1) previously associated with the capacity of killing hepatocytes. Conversely, $ICOSL^+$ MoMFs expressed CD9 and the Triggering Receptor Expressed on Myeloid cells-2 (TREM-2) that characterize NASH-associated macrophages (NAMs). ICOS deficiency strongly reduced $CD8^+$ T-cell expansion and prevented PD-1 upregulation. Such effect was also associated with a lowering in the expression of CD122, the β-chain component of both IL-2 and IL-15 receptors responsible for $CD8^+$ T-cell proliferation and survival.

CONCLUSIONS

Altogether, these data indicate that ICOS signals are critical for the expansion of auto-aggressive $CD8^+$ T-cells in NASH and suggest a possible interaction between these lymphocytes and NAMs, thus indicating ICOS/ICOSL dyad as a possible target for therapeutic interventions.

P011

THE ROLE OF INTERFERON-GAMMA IN AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE 1

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BACKGROUND-AIM

Autoimmune polyendocrine syndrome type 1 (APS-1) is a life-threatening, autosomal recessive syndrome caused by autoimmune regulator (AIRE) deficiency. In APS-1, self-reactive T cells escape thymic negative selection, infiltrate organs, and drive autoimmune injury. The effector mechanisms governing T-cell–mediated damage in APS-1 remain poorly understood.

METHODS

We examined whether APS-1 could be classified as a disease mediated by interferon-γ. We first assessed patients with APS-1 who were participating in a prospective natural history study and evaluated mRNA and protein expression in blood and tissues. We then examined the pathogenic role of interferon-γ using Aire$^{-/-}$Ifng$^{-/-}$ mice and Aire$^{-/-}$ mice treated with the Janus kinase (JAK) inhibitor ruxolitinib. On the basis of our findings, we used ruxolitinib to treat five patients with APS-1 and assessed clinical, immunologic, histologic, transcriptional, and autoantibody responses.

RESULTS

Patients with APS-1 had enhanced interferon-γ responses in blood and in all examined autoimmunity-affected tissues. Aire$^{-/-}$ mice had selectively increased interferon-γ production by T cells and enhanced interferon-γ, pSTAT1, and CXCL9 signals in multiple organs. Ifng ablation or ruxolitinib-induced JAK–STAT blockade in Aire$^{-/-}$ mice normalized interferon-γ responses and averted T-cell infiltration and damage in organs. Ruxolitinib treatment of five patients with APS-1 was associated with decreased levels of T-cell–derived interferon-γ, normalized interferon-γ and CXCL9 levels, and remission of alopecia, oral candidiasis, nail dystrophy, gastritis, enteritis, arthritis, Sjögren's-like syndrome, urticaria, and thyroiditis. No serious adverse effects from ruxolitinb were identified in these patients.

CONCLUSIONS

Our findings indicate that APS-1, which is caused by an autoimmune regulator deficiency, is characterized by excessive, multiorgan interferon-γ–mediated responses. JAK inhibition with ruxolitinib in five patients showed promising results.

P012

DEFINITION OF A THRESHOLD VALUE FOR THE KAPPA INDEX IN THE DIAGNOSIS OF MULTIPLE SCLEROSIS

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BACKGROUND-AIM

The Kappa Index is a biomarker of intrathecal immunoglobulin synthesis, suggested by numerous studies as a valid support in the diagnosis of multiple sclerosis (MS). However, there is no consensus among experts about the optimal threshold value. Our goal is to evaluate the diagnostic performance of the Kappa Index and to find an optimal threshold to discriminate between MS and non-MS patients.

METHODS

We included 118 patients (46 males, 72 females; mean age: 47.3 ± 17.3 years) with suspected MS referred to the University Hospital of Udine between November 2022 and January 2024. Patients were divided into 2 groups depending on the final diagnosis: the MS group (51 patients) and the non-MS group (67 patients). The assessment of oligoclonal bands (OCBs) via isoelectric focusing and immunofixation was followed by the determination of serum and cerebrospinal fluid (CSF) kappa free light chains (kFLCs) and albumin on the BN II nephelometric analyzer (Siemens, Germany) using the N Latex FLC Kappa and N Antiserum to Human Albumin assays. The limit of detection for kFLCs in CSF was 0,034 mg/L. Kappa Index was calculated as follows: (CSF kFLCs/serum kFLCs)/(CSF albumin/serum albumin).

RESULTS

The Kappa Index accuracy defined by area under the receiver operating characteristic curve (AUC) was higher than OCBs (94.4% vs. 88.2%). The optimal threshold, defined by applying the Youden Index, was 3.9; however, the diagnostic performance of this threshold value differed from that of the OCBs (sensitivity: 94.1% vs. 82.3%; specificity: 83.6% vs. 94.0%). A threshold of 6.9 had a specificity that was closer to OCBs (91.0%), while maintaining a good sensitivity (86.3%). Moreover, this threshold value showed a slightly better concordance with OCBs than the one defined by the Youden Index (91.5% vs. 88.9%).

CONCLUSIONS

Our study confirmed the validity of the Kappa Index as a biomarker for MS. We find the threshold value of 6.9 to be the best compromise between a good diagnostic performance and concordance with OCBs, considering the final goal of this marker is to be used in our laboratory in combination with the assessment of OCBs to help the clinician in the diagnostic decision.

P013

HYPOXIA-DEPENDENT BCR/ABL LOSS IN CHRONIC MYELOID LEUKEMIA CELLS IS DRIVEN BY EXTRACELLULAR VESICLES RELEASE

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BACKGROUND-AIM

Chronic Myeloid Leukemia (CML) is a myeloproliferative disease driven by a singular oncogene, BCR/Abl, which encodes a constitutively active tyrosine kinase. Despite the effectiveness of the tyrosine kinase inhibitors (TKi), the persistence of a subpopulation of TKi-resistant leukemia stem cells (LSCs) sustains the minimal residual disease. The LSCs are believed to persist in bone marrow niches, which are sites characterized by severe reduction in oxygen and nutrients, and are likely genetically BCR/Abl-positive but oncoprotein-negative. Such a hostile microenvironment may promote the biogenesis and release of extracellular vesicles (EVs), lipid bilayer-limited nanoparticles, exploited as biochemical messengers or for eliminating unwanted materials. Therefore, we aimed to study if such a mechanism might be used by CML cells to rapidly remove BCR/Abl during lack of oxygen.

METHODS

CML cells were subjected to severe hypoxia (0.1% O_2) for 96 hours to downregulate BCR/Abl expression. EVs were isolated from conditioned media via ultracentrifugation and analyzed through the Nanosight NS300. The isolated EVs morphology was visualized throughout the Transmission Electron Microscope and their cargo was analyzed via droplet digital PCR, while the biological effect was evaluated by flow cytometry, viability assay and quantitative PCR. The inhibition of EVs biogenesis and secretion was achieved by treating CML cells with Sulfisoxazole.

RESULTS

During the incubation in low oxygen conditions, mimicking in this way the stem cell niche microenvironment, we observed increased EVs secretion. Moreover, the EVs isolated in hypoxic conditions resulted loaded with high levels of BCR/Abl mRNA and capable of transferring their cargo to BCR/Abl-negative cells. Upon uptake of hypoxia-induced EVs, these cells demonstrated to become sensitive to TKi, phosphorylate CrkL, and increase proliferation rate. By inhibiting EVs biogenesis and secretion with Sulfisoxazole, an endothelin receptor antagonist, we were able to maintain high levels of BCR/Abl oncoprotein in CML cells subjected to hypoxia, resulting thereby susceptible to TKi.

CONCLUSIONS

EVs are typically secreted by all cells but their biogenesis is commonly enhanced by stress signals such as metabolic limitations. This mechanism was exploited by LSCs to rapidly “get rid” of BCR/Abl, facilitating entry into a quiescent status. Therefore, the inhibition of EVs biogenesis and secretion prevented BCR/Abl loss and thus led to the re-sensitization to TKi.

P014

NEW REGENERATIVE AND ANTI-AGING MEDICINE APPROACH BASED ON SINGLE STRAND ALPHA-1 COLLAGEN

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BACKGROUND-AIM

Over the past decade, regenerative medicine, particularly in skin treatment and rejuvenation, has seen significant advancements. This study explores the effectiveness of a new medical device, in treating various skin conditions, including aging, acne scars, and smoking-related effects. The device combines non-crosslinked high-molecular-weight hyaluronic acid (HMW-HA), human recombinant polypeptide of collagen-1 alpha chain, and carboxymethyl cellulose (CMC), aiming not only to hydrate but also to regenerate skin by promoting collagen production and improving skin elasticity and texture.

METHODS

The study involved 100 subjects divided into three groups based on their skin conditions. The treatment protocol included two injections of the medical device one month apart, with evaluations conducted at baseline, 30, and 60 days post-treatment using FACE-Q questionnaires and Antera 3D skin scanner measurements.

RESULTS

The results showed a significant improvement in skin quality, reduction in wrinkle depth, and an increase in skin elasticity and texture across all groups. Additionally, the study highlighted the safety and low risk of adverse reactions associated with the use of this new collagen formulation, making it a promising tool in regenerative medicine for skin treatment.

CONCLUSIONS

The innovative combination of ingredients in the medical device not only provides hydration but also stimulates both “in vitro” and in “vivo”, the skin's natural regenerative processes, leading to significant improvements in skin quality. The study's findings contribute to the field of regenerative medicine by demonstrating the effectiveness and safety of this new treatment approach for various skin conditions, offering a significant advancement in skin rejuvenation and treatment strategies.

P015

PLASMA TRANSTHYRETIN AND RETINOL BINDING PROTEIN IN CARDIAC AMYLOIDOSIS: AN ELECTROPHORETIC STUDY OF THEIR PATTERN

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BACKGROUND-AIM

Transthyretin cardiac amyloidosis (ATTR-CA) is caused by extracellular deposition of transthyretin (TTR), a plasma carrier of thyroxin and retinol-binding protein (RBP4). The disruption of the TTR tetrameric structure is the permissive step for the deposition of amyloid fibrils within extracellular matrix of cardiac and nervous tissues. A pharmacological strategies used to treat this disease is based on tafamidis, a stabilizer of TTR tetramers.
Aim of this study was to develop a method to characterize circulating TTR and TTR/RBP complexes in plasma samples of ATTR-CA patients before and after administration of tafamidis.

METHODS

Plasma Li-heparin samples from healthy subjects (n= 30) and patients (n=30) with ATTR-CA were studied. Healthy subjects were selected from the CATCH study, a Tuscany screening study performed on elderly subjects (≥65yrs). ATTR-CA patients eligible for tafamidis treatment were selected to the Cardiology Division of the Fondazione Toscana Gabriele Monasterio (FTGM). ATTR-CA patients were re-assessed at day 14, 30, 90, 180 after starting tafamidis assumption. Total plasma TTR and RBP4 were quantified by nephelometry (Atellica, Siemens). Samples were separated on a native 4–20% Tris-Gly polyacrylamide gel associated with western blot.

RESULTS

The patterns of TTR circulating fractions were qualitatively similar between ATTR-CA patients at T0 and healthy subjects. In both groups, the most represented forms were: TTR dimers or trimers (37-50kDa), tetramers complexed with 1 (80kDa) or 2 RBP4 protein (100KDa), and high molecular weight (MW) aggregates (>150kDa). TTR monomers were never detectable. RBP4 protein was detectable only in association with TTR tetramers. Following tafamidis treatment dimers and trimers, detectable at T0, were progressively lost during tafamidis treatment.

CONCLUSIONS

TTR tetramers mainly exist in association with RBP4 and in equilibrium with high and low MW degradation forms. When retinol is delivered to tissues, RBP4 affinity for TTR lowers and it may dissociate favouring the disassembling of tetramers into trimers, dimers, and monomers. The latter have a high tendency to self-aggregate into the high MW TTR fractions. The technique also shows an appreciable effect of tafamidis treatment on the stabilization of circulating TTR tetramers complexed with RBP. The study of circulating TTR fractions can expand our knowledge on mechanisms provoking its destabilization even when not mutated.

P016

BCLAF1 FUNCTIONALIZATION AGAINST NEUROBLASTOMA

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BACKGROUND-AIM

Neuroblastoma (NB) is the most common extracranial solid childhood tumor. It arises from impairment of neural crest (NC)-derived cell differentiation during sympathetic nervous system development. NB characterized by wide genetic, epigenetic, and phenotypic heterogeneity that impact upon clinics in term of diagnosis, therapeutic response and survival (PMID: 30116755; 10985139). Despite extensive studies and trials, outcomes have been stable, and the acquisition of multidrug resistance remain the major obstacle to the successful treatment of NB. Our recently published in acute myeloid leukemia (AML) (PMID: 28216661) and new preliminary data provided the evidence of functional role for module miR- 194-5p/BCLAF1 in intracellular and extracellular compartments in NB (PMID: 28216661). We defined BCLAF1 as novel intracellular player and extracellular messenger in NB, influencing tumorigenicity and drug-resistance.

METHODS

NB cell lines,primary samples,mouse models;PCR&RT-PCR;Western blot;cell cycle,proliferation,death and differentiation tests;IF;RNA-scope;RNA-seq,ChiP-seq,LC-MS

RESULTS

Our data show BCLAF1 over-expression and its correlation to MYCN amplification in NB cell lines and primary samples. We characterized BCLAF1 epigenetic modulation by histone deacetylase inhibitor, SAHA, specifically on its oncogenic isoform down-regulation. Interestingly, we also found its over-expression and modulation in small vesicles strengthening the use of BCLAF1 as a new circulating biomarker. Our results support BCLAF1 potential role in differentiation commitment defects in NB. During lineage commitment of normal human adrenal medulla BCLAF1 is almost absent in most differentiated cell populations. Interestingly, its contribution to neural commitment is also confirmed through BCLAF1 expression by specific subpopulation of mouse sympathoblasts in early sympathetic ganglia and by ATRA in NB cell lines. These findings not only reveal a double task of BCLAF1 in NB aberrant proliferation and death resistance but also its contribution to the lineage commitment defects of NC cells. Clearly, NB complex clinical and molecular landscape cannot be precisely described by a single marker, but we speculate about the integration of BCLAF1 into clinical consideration as potential biomarkers for an identification of early cancer and the selection of the most effective therapy approaches, also epigenetic combined.

CONCLUSIONS

We suggest BCLAF1 targeting for rationally designed diagnostic tools and new therapeutic approaches in NB

P017

SIMVASTATIN AND DOXORUBICIN CO-TREATMENT REDUCES DOX-INDUCED OXIDATIVE STRESS AND APOPTOSIS

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BACKGROUND-AIM

Doxorubicin (Dox)-induced cardiotoxicity is a major cause of long-term morbidity and mortality among cancer survivors. Recent studies described Dox-induced cardiotoxicity as a continuous phenomenon, starting with myocardial cell damage, followed by a progressive functional decline, which gradually leads to overt heart failure. Evidence recognized a central role of oxidative stress and inflammation, which appear to be mutually related. The discovery of therapeutic strategies able to act in the early phase are needed. Drug repurposing may represent a promising approach. Simvastatin (Sim), a HMG-CoA reductase inhibitor, showed a remarkable cardioprotective effect in view of its anti-oxidant and anti-inflammatory properties. Here, we investigated the cardioprotective role of Sim on Dox-induced acute cardiotoxicity in an in vitro model.

METHODS

Human cardiomyocytes (HCM) were pre-treated with Sim (5,10,20 μM) for 4h and then Dox (1 μM) was added. The effects of Sim co-treatment on Dox-induced oxidative stress and apoptosis were evaluated at different time points by MTS, Real-Time PCR and FACS analyses.

RESULTS

MTS assay results demonstrated that Sim co-treatment significantly ($p<.05$) reduced Dox-induced cell death. Real time-PCR results reported a significantly ($p<.05$) decrease of ErbB2 and ErbB3 expression after both Dox alone and Sim co-treatment. FACS analyses showed that Sim co-treatment significantly ($p<.05$) reduced both cytosolic and mitochondrial Dox-induced ROS production, as well as cytochrome c release ($p<.01$) and mitochondrial membrane depolarization ($p<.005$). A significant ($p<.05$) reduction was observed also in Dox-induced Cx43 phosphorylation on Ser368 residues increased levels. Furthermore, a significant ($p<.05$) reduction in Dox-induced SOD2 overexpression has been reported in Sim co-treated cells.

CONCLUSIONS

Previous studies reported how, after Dox treatment, cardiomyocytes try to reduce the spread of death signals to neighboring cells and increase defense mechanism against free radical. Thus, in line with previous literature, we can hypothesize that Sim co-treatment reduces Dox-induced oxidative stress and apoptosis, leading HCM to reduce defense mechanisms, representing a possible strategy to protect HCM against Dox-induced damage.

P018

THE LONG PENTRAXIN PTX3 SERVES AS AN EARLY PREDICTIVE BIOMARKER OF CO-INFECTIONS IN COVID-19

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BACKGROUND-AIM

COVID-19 clinical course is highly variable and secondary infections contribute to COVID-19 complexity. Early detection of secondary infections is clinically relevant for patient outcome. Procalcitonin (PCT) and C-reactive protein (CRP) are the most used biomarkers of infections. Pentraxin 3 (PTX3) is an acute phase protein with promising performance as early biomarker in infections. In COVID-19 patients, PTX3 plasma concentrations at hospital admission are a strong independent predictor of poor outcome. In this study, we assessed whether PTX3 contributes to early identification of co-infections during the course of COVID-19.

METHODS

We analyzed PTX3 levels in COVID-19 patients with (n=101) or without (n=179) community or hospital-acquired fungal or bacterial secondary infections (CAIs or HAIs), comparing it with classical biomarkers used by clinicians, i.e., PCT and CRP.

RESULTS

PTX3 plasma concentrations at diagnosis of CAI or HAI were significantly higher than those in patients without secondary infections. In a longitudinal analysis, PTX3 peaked at admission and significantly increased again at the co-infection time-point. In patients surviving the secondary infection, the protein concentration significantly decreased after co-infection diagnosis, whereas in non-survivors, only a trend of decrease was observed. In selected patients, for whom we had serial PTX3, PCT and CRP measurements in a time period close to the HAI diagnosis, PTX3 plasma levels almost doubled few days before the diagnosis, when PCT and CRP concentration were still in the range of moderate increase or normality. Compared to PCT and CRP, the increase of PTX3 plasma levels was associated with the highest hazard ratio for CAIs and HAIs (aHR 11.68 and 24.90). In univariable and multivariable Cox regression analysis, PTX3 was also a strong predictor of 28-days mortality or intensive care unit admission of patients with potential co-infections, faring more pronounced than CRP and PCT.

CONCLUSIONS

PTX3 is a promising predictive biomarker for early identification and risk stratification of COVID-19 patients with co-infections.

P019

INDOLE-3-ALDEHYDE: THE STORY OF A POSTBIOTIC FROM BENCH TO BEDSIDE

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BACKGROUND-AIM

Postbiotics are the repertoire of metabolites produced in the fermentation process of dietary components (mainly fibers and polyphenols, but also complex carbohydrates, proteins, and lipids), as well as the endogenous components generated by bacteria-host interactions that influence human health. They are recognized as promising for providing therapeutic benefits, including eubiosis maintenance at mucosal surfaces.

METHODS

We describe here the multifunctional activity of the postbiotic indole-3-aldehyde (3-IAld) in preclinical human and murine models of infections and inflammatory diseases.

RESULTS

By resorting to targeted delivery technologies, 3-IAld administration via dry powder inhalation or oral delivery was an efficient strategy to restore immune and microbial homeostasis in preclinical models of brain, liver, lung and gut inflammation. In vitro, 3-IAld moonlighted as a metabolite and signaling molecule by acting as a rapidly metabolized ligand of the human aryl hydrocarbon receptor (AHR) and pregnane activated receptor (PXR), both receptors known to affect mucosal and microbial homeostasis at different body sites. More recently, 3-IAld administration increased resistance to infections and prevented the occurrence of inflammatory dysbiosis in anti-leukemic- or checkpoint inhibitors-treated animals.

CONCLUSIONS

As 3-IAld production is reduced in a variety of human diseases, including atopic dermatitis, inflammatory bowel and lung diseases, these findings suggest that replacement therapy with 3-IAld may be of benefit in a variety of human inflammatory diseases. The recently obtained orphan drug designation in a primary immunodeficiency is a step forward in this direction.

P020

THE BONE MARROW NICHE MICROENVIRONMENT GOVERNS LEUKAEMIA STEM CELLS' STEM CELL POTENTIAL AND QUIESCENCE MAINTENANCE

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BACKGROUND-AIM

Chronic Myeloid Leukaemia (CML) is a stem cells-driven myeloproliferative disease which occurs in hematopoietic stem cells due to the reciprocal t(9;22) translocation, which culminates with the expression of BCR/Abl tyrosine kinase. Albeit the great efficacy of Tyrosine Kinase inhibitors (TKi), past and current available drugs are not curative yet. The bone marrow (BM) stem cell niche (SCN) microenvironment features such as very low oxygen tension, secreted cytokines (Bone Morphogenetic Proteins (BMPs) above all and cells' adherence to the niche, are critically involved in the maintenance of Leukaemic Stem Cells' (LSCs) dormancy and persistence, thus favouring the minimal residual desease. This work aims to unravel how very low oxygen-related LSCs metabolism, as well as the interaction with the endosteal niche coupled to BMPs secretion, modulate the maintenance of CML LSCs stem cell potential, alongside dormancy.

METHODS

Two immortalized CML cell lines (K562 and KCL22) were cultured at very low oxygen atmosphere to address the role of very low oxygen-related metabolism in LSCs persistence. Post hypoxic incubation, cells were transferred into normoxic secondary non-selective cultures to evaluate their stem cell potential maintenance, or lysed to assess BCR/Ablprotein expression and signalling. Secondly, a BM-like 3D model was used to inquire how CML LSCs dormancy is fostered, in long-term treatment, due to CML cells' adherence to the endosteal niche and the secretion BMP-4.

RESULTS

Hypoxia forced LSCs to almost cease BCR/Ablprotein expression during their incubation, with a consequent loss of their oncogene-addiction and thereby to be targetable by TKi. This event, mainly dependent on glucose consumption and lactate extrusion, is regulated by glutamine availability within the niche. After a variable lag-phase lasting period, cells were capable to re-gain BCR/Ablprotein expression yielding to the repopulation of the normoxic cultures. Secondly, we outlined how BMP-4 promotes LSCs' rapid commitment in a deep G0 quiescent state under TKi treatment. Moreover, the 3D system has revealed a block of cells into the G1 and a significant decrease in the G2-M cell cycle phases, when subjected to TKi treatment.

CONCLUSIONS

Thus, we conclude that the loss of LSCs reliance upon BCR/Ablprotein expression and signalling, alongside the fast commitment towards a deep quiescent state, are key events involved in both LSCs' refractoriness to TKi and persistence within the SCN microenvironment.

P021

THE IMPACT OF A LONG-TERM WESTERN DIET INTERVENTION ON THE CARDIOVASCULAR SYSTEM OF SEDENTARY FEMALE LONG-EVANS RATS

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BACKGROUND-AIM

Obesity is a pathological condition characterized by abnormal or excessive fat accumulation. This condition is associated with many health issues like liver diseases, diabetes, hypertension and cancer. Obesity impact the cardiovascular system significantly leading to pathological cardiac remodeling, indeed obese people have double the risk of developing heart failure. In recent years, obesity has become significantly more prevalent worldwide with many calling this phenomenon the “obesity epidemic”.

Many attribute the rise of obesity incidence to the western style diet (WD) which is characterized by high intake of sugar and fat. The aim of this work was to assess the effect of a WD on the cardiovascular system.

METHODS

Female Long-Evans rats were divided into 4 groups: control group (CTRL) fed a purified “control to Surwit” diet, high sugar (HS) diet (water with fructose and sucrose), high fat “Surwit” diet + 1% cholesterol (HF), high fat high sugar diet (HFHS) (Surwit diet and sugar water). Dietary interventions have been carried out for 8 months, starting at the 5th month of age.

RESULTS

The dietary intervention caused significant weight gain in the HF group compared to CTRL of this model. Visceral adipose tissue (VAT) was significantly increased in the HF and HFHS groups. Liver microvesicular steatosis was observed only in the HF and HFHS groups, while liver inflammation was observed in HS, HF and HFHS groups but not in the CTRL group. Cardiac fibrosis was significantly higher only in the HF diet group compared to the CTRL.

Serum free cholesterol, triglyceride and phospholipid levels were increased in all dietary interventions with respect to CTRL. Serum alanine amino transferase and alkaline phosphatase were both elevated in HF and HFHS groups compared to CTRL, while albumin was significantly reduced in HF and HFHS rats with respect to controls.

Metabolomics was carried out in left ventricle (LV), heart apex and aorta (aortic arch and descending aorta). Glutamate, glutathione and guanosine were altered in the descending aorta while carnitine, tryptophan, glutamine and S-methyl-5-thyoadenosine were altered in the aortic arch.

In the left ventricle changes in betaine and choline were observed as well as taurine and other metabolites. Taurine and choline were also found altered in the heart apex.

CONCLUSIONS

In summary, our study demonstrates increased VAT accumulation, cardiac fibrosis and alteration of cardiac metabolism in our Western diet fed rat model.

P022

TARGETING OF THE MITOCHONDRIAL E3-LIGASE MARCH5 ENHANCES VENETOCLAX SENSITIVITY OF MULTIPLE MYELOMA CELLS VIA MITOCHONDRIAL FUSION AND OXPHOS IMPAIRMENT

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BACKGROUND-AIM

Accumulating evidence suggests imbalance of mitochondrial fission/fusion events in multiple myeloma (MM) due to defective post-translational regulation of its major drivers. Herein, we investigated the functional role of the mitochondrial E3-ubiquitin ligase Membrane Associated Ring-CH-Type Finger 5 (March5), an emerging regulator of mitochondrial dynamics, on MM cell growth, metabolic reprogramming and drug resistance.

METHODS

March5 mRNA expression was retrieved from GSE47552 and GSE116294 datasets. RNA sequencing was performed by the Illumina platform. Mitochondrial structure was assessed by TEM. Cell viability was assessed by Cell Titer Glo. March5 overexpression (OE) was carried out by lentiviral vectors, while silencing using siRNAs. Cycloheximide chase assays were performed to evaluate Mitofusin 2 (MFN2) protein stability. Mitochondrial oxygen consumption rate (OCR) was measured via Oroboros respirometry.

RESULTS

March5 gene was found progressively upregulated in MM starting from MGUS and SMM conditions, associating with worse OS and PFS in the CoMMpass dataset. Silencing of March5 resulted in elongated mitochondria, as assessed by TEM, indicating a shift towards mitochondrial fusion. Consistently, March5 silencing increased the protein expression of the fusion driver MFN2, while March5 OE reduced MFN2 protein half-life. RNA-seq analysis of March5-depleted cells showed the down-regulation of pathways involved in mitochondrial ETC coupled to ATP synthesis, previously associated to the onset of venetoclax resistance. Accordingly, silencing of March5, as well as MFN2 OE, enhanced venetoclax sensitivity, while March5 OE dampened venetoclax ant-MM cytotoxicity. At a metabolic level, March5 silencing or venetoclax treatment reduced, while March5 OE increased OXPHOS antagonizing venetoclax metabolic activity.

CONCLUSIONS

These data indicate that March5 negatively regulates the mitochondrial dynamics network, thus representing a new target to restore venotoclax sensitivity in MM.

P023

ACQUIRED RESISTANCE TO KRASG12C INHIBITORS VIA AUTOCRINE SECRETION OF STROMAL PROTEINS IN NON-SMALL CELL LUNG CANCER

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BACKGROUND-AIM

The advent of $KRAS^{G12C}$ inhibitors has represented a pivotal advance in Non-Small Cell Lung Cancer (NSCLC) treatment. The FDA-approved Sotorasib and Adagrasib demonstrated their efficacy on $KRAS^{G12C}$ mutation in the clinical setting. Nevertheless, the emergence of acquired drug resistance has been documented. The resistance mechanisms are varied and not yet fully understood; thus, exploring new therapeutic strategies to fight them is necessary.

METHODS

Resistant cell clones were generated from $KRAS^{G12C}$ mutated H23 cells treated with increasing doses of Sotorasib and Adagrasib over six months. NGS sequencing was performed with a TSO500 panel. Gene expression was investigated by RNA sequencing, GSEA analysis, and the genes of interest were validated by RT-PCR. Protein expression and phosphorylation were evaluated by western blotting. The effect of drug was assessed by SRB assay.

RESULTS

We isolated two resistant clones from the H23 cell line for each KRAS inhibitor, with a significantly higher IC_{50} and phosphorylated ERK1/2 levels than the parental cell line. We confirmed the presence of $KRAS^{G12C}$ and the absence of both KRAS and new driver alternative mutations through NGS analysis. By RNAseq approach, we identified more than 300 genes differentially expressed between the resistant clones and the parental cells treated with the KRAS inhibitors. Three genes (COL4A2, CXCL1 and HGF) were subsequently identified as putative responsible for resistance and their expression levels were confirmed by RT-PCR experiments. CXCL1 belongs to the alpha-chemokine subfamily, HGF and COL4A2 are reported to activate MET and FAK signaling. The resistant clones exhibited increased phosphorylation of both these proteins compared with the sensitive cells. The monotherapy with crizotinib (MET inhibitor), defactinib (FAK inhibitor) or SB225002 (the inhibitor of CXCR4-CXCL1 binding) failed to revert the resistance to KRAS targeting agents. Experiments of drug combination are in progress.

CONCLUSIONS

Our data indicate the presence of concomitant resistance mechanisms based on the secretion of HGF and CXCL1 and on COL4A2 overexpression. The concurrent use of drugs may represent an effective strategy for reverting the resistance to $KRAS^{G12C}$ inhibitors.

P024

THE STEAROYL-COA DESATURASE SCD1 DRIVES FERROPTOSIS RESISTANCE IN PROTEASOME INHIBITOR-REFRACTORY MULTIPLE MYELOMA

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BACKGROUND-AIM

Ferroptosis gene signatures endowed with clinical significance have been identified in multiple myeloma (MM) patients, although the therapeutic potential of ferroptosis-inducing strategies remains unexplored. Herein, we investigated the molecular mechanisms underlying the responsiveness of proteasome inhibitor (PI)-sensitive or -resistant MM cells to pro-ferroptotic drugs.

METHODS

PI-resistant (AMO-BZB, H929-BZB, H929-CFZ) cell lines were generated through continuous exposure of parental cells to bortezomib or carfilzomib. MUFA levels were determined by targeted lipidomics. Cell viability was assessed by Cell Titer Glo assay, while apoptosis by Annexin V/7AAD staining. Lipid peroxides were determined by Bodipy-C11 FACS staining. Mitochondrial oxygen consumption rate (OCR) was measured via Oroboros respirometry. Subcutaneous xenografts with AMO-BZB cells were used for in vivo studies.

RESULTS

PI-resistant MM cells were found to be more sensitive to pro-ferroptotic drugs (Erastin, Rsl3) as compared to their parental counterpart. Since high levels of MUFA have been proven to induce resistance to ferroptosis, we investigated the expression pattern and the functional role of SCD1, the rate-limiting enzyme in MUFA production. PI-resistant cells expressed higher levels of MUFA and SCD1 at mRNA and protein levels, while only PI-sensitive cells down-regulated SCD1 upon PI treatments. Of note, enforced expression of SCD1 in PI-sensitive cells conferred growth advantage and triggered resistance to both PI and pro-ferroptotic agents. Pharmacological targeting of SCD1 via CAY10566 or A9399572 reduced OXPHOS, triggering apoptosis and ferroptosis in PI-resistant cells, in vitro as well as in vivo in a bortezomib-resistant xenograft model of MM.

CONCLUSIONS

These data indicate that SCD1 overexpression drives resistance of PI-refractory MM cells to ferroptosis, providing the first preclinical rationale for the clinical investigation of SCD1 inhibitors in replased/refractory MM patients.

P025

PIN1 STERIC HINDRANCE ON WWP2 DEGRON SITE: A NOVEL MOLECULAR REGULATION OF NOTCH3 IN OVARIAN CANCER

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BACKGROUND-AIM

Ovarian Cancer (OC) is one of the most lethal female-related disease malignancies. Its curative and survival trends have not changed over the past decades because of the insurgence of recurrence connected with resistance to platinum (PT)-based chemotherapies. Therefore, finding novel targetable biomarkers is required. Given its role in tumorigenesis in OC, the Notch3 (N3) signalling pathway has emerged as promising candidate for novel specific targeted therapies. Consequently, the prediction of the interaction between N3 and its regulators, through the exploitation of the N3 post-translational modification (PTMs), is emerging as an innovative approach. In this scenario, the appealing candidates to fine-tune N3 stability are the peptidyl-prolyl cis/trans isomerase Pin1, previously identified in our lab as a N3-positive regulator, and the E3 ligase WWP2, known to ubiquitinate N3 in OC. Our preliminary results supported the hypothesis that Pin1 is able to hinder the WWP2 activity due to the close proximity of their consensus sites in the N3 intracellular domain (N3ICD). Therefore, we dissect the molecular mechanism underlying: 1. The WWP2 functional activity on N3ICD protein, and 2. The Pin1-WWP2 potential antagonistic interplay on N3ICD.

METHODS

We performed: mutagenesis of N3 protein, co-transfection experiments and co-immunoprecipitation assay.

RESULTS

First, we observed that WWP2 is involved in the N3ICD poly-ubiquitination. Additionally, to fully understand whether and how Pin1 and WWP2 competition on N3ICD influences its degradation/stability, we performed i) Co-immunoprecipitation assays using N3ICD mutants in Pin1 or WWP2 consensus motifs, and ii) Ubiquitination assays in presence or absence of Pin1 protein. We observed that Pin1 steric hindrance hamper WWP2-N3 interaction on WWP2 degron site. Moreover, using a peptide, named "TAT-NOTCH3-TAD", able to recognize and interact with Pin1, as it displays only the Pin1 consensus motif, we demonstrated that increasing doses of TAT-NOTCH3-TAD peptide blocks the Pin1 competition, thus restoring WWP2 activity on N3ICD.

CONCLUSIONS

Overall, these data will help us to deepen our knowledge of N3 post-translational regulation, via Pin1-WWP2 competition, finally identifying new molecular targets aimed at developing novel therapeutic strategies.

P026

EXPLORING THE ROLE OF PRDM GENES IN GLIOMA PATHOGENESIS AND THERAPEUTIC POTENTIAL

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BACKGROUND-AIM

Gliomas are the most common primary tumors of the central nervous system, classified by histopathological analysis into four grades based on their malignancy (WHO Grade I, II, III, IV). Low-grade gliomas (LGGs), which include astrocytomas, oligodendrogliomas, and oligoastrocytomas, are well-differentiated and have a relatively favorable prognosis, with a 5-year survival rate of approximately 60%. Unfortunately, most LGGs progress to glioblastoma (GBM) within 5 to 10 years. GBM is the most common and aggressive primary brain tumors, characterized by difficult treatment, poor clinical prognosis, and high lethality.
Despite the progress and the significant advances in the understanding of the molecular and genetic alterations that promote GBM onset have enabled novel available therapeutics, effective treatment options are limited. Therefore, it is significant to explore new genes and molecular markers for the treatment of different glioma grades. PR (PRDI-BFI and RIZ) domain containing (PRDM) proteins have been shown to play a pivotal role in several human cancer types. However, their function in gliomas remains to be elucidated.

METHODS

In this study, we have used The Cancer Genome Atlas (TCGA) datasets on GEPIA2 platform to investigate the possible role of PRDM genes in LGG and GBM through bioinformatics analysis. qRT-PCR evaluation on different GBM primary cell lines (n= 10 cell lines), derived from tumor brain specimens, was carried out to confirm expression data.

RESULTS

A first in silico analysis of the PRDM gene expression by TCGA- LGG and -GBM datasets on GEPIA2 platform revealed a significant overexpression of PRDM1, PRDM5 and PRDM11 and a downregulation of PRDM2, PRDM8 and ZNF408 genes in both LGG and GBM compared to normal tissues. qRT-PCR analysis on cell lines derived from tumor brain specimens confirmed the obtained findings. Subsequent detailed analyses of the different PRDM2 transcripts have provided some novel insights on the dual behavior of this gene also in brain malignancies.

CONCLUSIONS

Further studies are needed to confirm these preliminary results and to delve the peculiar role of all PRDM genes in glioma and their involvement in pivotal signaling pathways implicated in gliomas. The characterization of PRDM roles in glioma could define new targetable players responsible of therapy resistance and relapse.

P027

SCREENING OF HIGH-RISK PREGNANCIES FOR DOWN SYNDROME USING QUADRUPLE TEST AT A TERTIARY CARE CENTER OF NEPAL

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BACKGROUND-AIM

Down syndrome (DS) is the most common cause of developmental delay and accounts for 15-30 percent of individuals with intellectual disabilities. No finite published data suggests the prevalence of DS in Nepalese children. There is no definitive protocol from the government or professional bodies regarding screening, though the practice for screening DS is increasing both in the public and private sectors. This study aimed to screen high-risk pregnant women (age ≥ 30 years) by quadruple biochemical test in the second trimester for Down syndrome and other chromosomal abnormalities and to evaluate the sensitivity and accuracy of the second-trimester screening (quadruple test with genetic sonogram) for trisomy 21 as compared to biochemical testing.

METHODS

This prospective observational study was conducted in the Department of Clinical Biochemistry in collaboration with the Department of Obstetrics and Gynecology, Institute of Medicine, TUTH, Maharajgunj, Kathmandu, Nepal. Pregnant women at 15-21 weeks of gestation were enrolled. Quadruple test (Alpha-fetoprotein (AFP), beta-human gonadotrophin (hCG), unconjugated estriol (UE3), and Inhibin-A in the laboratory using the technology of Chemiluminescence micro particle two-step immunoassay. Risk estimation using values of hormone levels and the resulting MoM was done using PRISCA 5.0.2.37.

RESULTS

256 high-risk pregnancies were screened for trisomy 21, trisomy 18, and neural tube defects. The mean age of the patient was 33.65 ± 3.71 years. Out of 256 patients, 24 (8%) patients were identified as high risk for trisomy 21, 6 (1.96%) patients for Trisomy 18, and 6 (1.96%) patients for neural tube defects. Multiples of Median (MOM) for AFP in high-risk pregnancy were (1.06 ± 0.57; Sensitivity: 43.8% and specificity: 47.2 %), B-HCG (1.27 ± 0.59; Sensitivity: 87.5% and specificity 71.9%), UE3 (0.86 ± 0.45; Sensitivity: 31.3% and specificity 30.2%) and Inhibin (2.67 ± 1.16; Sensitivity: 81.3% and Specificity: 85.4%) respectively.

CONCLUSIONS

Second-trimester quadruple test provides an effective screening tool for Down syndrome in the Nepalese population. Quadruple tests combined with sonograms can lower the rates of unnecessary amniocentesis in high-risk populations.

P028

THERAPEUTIC TARGETING OF MITOCHONDRIAL METABOLISM BY P2X4 RECEPTOR INHIBITION AND AMINO ACID RESTRICTION IN RENAL CARCINOMA MODELS.

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BACKGROUND-AIM

Background. Large-scale metabolomic data have associated metabolic alterations with the pathogenesis and progression of renal carcinoma and have correlated mitochondrial activity with poor survival in a subset of patients. Lysosomes are intracellular Ca2+ hubs that are essential for membrane trafficking and signaling. The lysosomal purinergic receptor 4 (P2XR4), an ATP/Ca2+ pump, plays a key role in energy flux. Furthermore, dietary restrictions have been reported to potentially modulate tumor metabolism. In this study, we investigated the role of P2XR4 inhibition and amino acid (AA) restriction in metabolic and energy dynamics in clear cell and translocation RCC models.

METHODS

Methods. Seahorse experiments, immunoflurescence and fluorescence cell sorting, genetic silencing, and pharmacological inhibition were utilized to assess the role of P2XR4 and AA in regulating mitochondrial function. Patient-derived organoids and murine xenograft models were used to demonstrate the impact P2XR4 inhibition and AA restriction.

RESULTS

Results Our data suggest that oxo-phosphorylation is the main source of tumor-derived ATP in a subset of ccRCC cells but in all the tRCC cells assessed. Mitochondrial function inhibition failure induced by pharmacological inhibition or P2XR4 silencing was associated with increased oxygen radical species, and changes in mitochondrial permeability. Amino acid restriction was associated with decreased oxidative phosphorylation in RCC models with baseline elevated mitochondrial function. The results from combining amino acid restriction and P2X4R inhibition are ongoing and will be presented.

CONCLUSIONS

Conclusion: Overall, our preliminary results suggest that the perturbed mitochondrial activity induced by P2XR4 inhibition and amino acid restriction may represent a new therapeutic strategy for a subset of RCC patients

P029

INHIBITION OF APE1 REDOX ACTIVITY AS NOVEL STRATEGY FOR THE TREATMENT OF INFLAMMATORY-BASED INTESTINAL DISEASES

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BACKGROUND-AIM

Inflammatory Bowel Diseases (IBDs) are chronic disorders characterized by inflammation and epithelial injury of the gastrointestinal (GI) tract. Therapeutic strategies in IBDs aim at modifying the immune response of the GI tract. Recently, it has been demonstrated the involvement of apurinic/apyrimidinic endodeoxyribonuclease 1 (APE1) in IBDs. APE1 is a DNA repair protein operating on lesions mainly due to oxidative stress, during the base excision repair pathway. Moreover, APE1 is an essential redox coactivator of several transcription factors regulating several biological processes including angiogenesis, inflammation, and proliferation. Interestingly, APE1 redox activity resulted to be increased in tissues resected from IBD-affected patients whereas increased expression of APE1 was observed in colon sections from rats affected by colitis induced with dextran sodium sulfate. Furthermore, data obtained in a mouse model of spontaneous chronic colitis have demonstrated that the use of the redox inhibitor of APE1 mitigates the consequences of the intestinal dysfunction. Together these observations allow to hypothesize that APE1 is involved in the pathophysiology of GI inflammation and could be used as a promising target for IBDs therapy.

METHODS

SW480 cells was stimulated with LPS in the presence or absence of the APE1 redox inhibitor APX2009 in combination with Infliximab and the drug's efficacy was evaluated focusing on the viability and on inflammatory phenotype.

RESULTS

We found that APX2009 affects SW480 cell metabolism without impairing cell viability. Moreover, APX2009 blocks LPS-induced activation of IL-8 production in a dose-dependent manner showing a synergic effect with Infliximab on LPS-induced IL-8 gene expression inhibition.

CONCLUSIONS

All these data are suggestive of the use of APE1 redox inhibitor as a novel strategy for the treatment of inflammatory-based intestinal diseases, alone and in combination with the anti-TNF-α strategies already used.

P030

TARGETING PIN1 TO INDUCE BRCANESS PHENOTYPE IN HIGH-GRADE SEROUS OVARIAN CANCER.

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BACKGROUND-AIM

High-grade serous ovarian cancer (HGSOC) is considered one of the deadliest female-related diseases since the prognosis is still poor despite the clinical advancements. The assessment of BRCA/Homologous-Recombination (HR) deficiency mutational status has considerably ameliorated the current treatments given that HR defective patients display higher sensitivity to platinum-based chemotherapies and Poly (ADP-ribose) polymerase inhibitors (PARPi)-based maintenance therapies. Nonetheless, the finding of novel therapeutic targets remains an urgent clinical need. In this scenario, the peptidyl-prolyl cis/trans isomerase Pin1 has emerged as a promising candidate given that it has been demonstrated that its inhibition resulted in a condition resembling BRCA1/2 germline mutation (known as BRCAness) in BRCA-proficient cells in breast cancer. Nevertheless, whether this occurs also in HGSOC is still unknown. Taken together, the present study aims at evaluating Pin1 as an actionable vulnerability in HGSOC with the goal of increasing the clinical efficacy of platinum drugs- and PARPi-based therapies.

METHODS

BRCA1-proficient established HGSOC cell lines were used. We performed: 1. in silico analysis on protein data collected from HGSOC-bearing patients; 2. lentiviral transductions to stably over-express doxycycline-inducible shRNA-Pin1; 3. in vitro studies such as co-immunoprecipitation assays, transient transfections (siRNA-Pin1), pharmacological treatments (ATRA, KPT-6566, Carboplatin), and cell viability; and 4. in vivo experiments: xenografts in NSG mice.

RESULTS

We documented a significant direct correlation between Pin1 and BRCA1 protein expression levels in a cohort of HGSOC-bearing patients. Furthermore, either genetic or pharmacological Pin1 targeting reduces BRCA1 protein expression, finally resulting in Carboplatin sensitization in vitro and in vivo. Thus, these observations corroborate our hypothesis that it is possible to induce BRCAness via Pin1 inhibition in HGSOC.

CONCLUSIONS

All in all, our findings reveal a promising treatment target by which platinum drugs and PARPi could benefit a wider range of BRCA1-proficient patients. Interestingly, this strategy may also impact the clinical care of BRCA1-mutated women where BRCA1 expression is restored during the progression of the disease.

P031

SENOLYTIC TESTING AND DEVELOPMENT AT THE GERIATRIC MOUSE CLINIC

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BACKGROUND-AIM

Geroscience, the interdisciplinary field at the intersection of aging and chronic disease research, aims to understand the fundamental mechanisms of aging and to target these mechanisms for the development of new therapies for age-related chronic diseases. Longitudinal and interventional studies in mice play a crucial role in advancing Geroscience.

METHODS

We will show an overview of ongoing and planned longevity interventions conducted at the Geriatric Mouse Clinic, focusing on innovative strategies to target senescent cells including genetic- and peptide-based senolytic drugs. We will focus on the current development of a new candidate senolytic drug identified by the combination of a virtual screening followed by “in vitro” selection.

RESULTS

This compound, named K5, display a unique dual action as xanthinic phosphodiesterase (PDE) and Heat shock protein 90 (HSP90) inhibitor. K5 demonstrated senolytic activity across various cellular senescence models, including human fibroblasts, mesenchymal stem cells, and breast cancer cells. It was also effective in vivo, extending lifespan in Drosophila and reducing senescence markers and frailty in geriatric mice.

CONCLUSIONS

In conclusion, senolytic testing in geriatric mice is powerful and complementary to other models, for developing effective treatments to be translated in geriatric settings. These models provide a broad representation of the aging process, ensuring that new therapies are both effective and safe for elderly populations. Clinical trials involving older adults with comorbidities are increasingly recognized as necessary, and a similar approach should be adopted in preclinical research to comprehensively evaluate therapeutic interventions prior to clinical advancement.

P032

STUDY OF THE MICRORNA-BASED MECHANISM IN A NOTCH3-INDUCED T-ALL MODEL

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BACKGROUND-AIM

T-cell acute lymphoblastic leukemia (T-ALL) arises from transformed T-cell progenitors, with Notch1 mutations and Notch3 overexpression in around 60% of cases, but occasional Notch3 mutations have been detected. Notch receptors guide T-cell maturation within the thymus through the CXCL12-CXCR4 axis that promotes chemotaxis, survival, and proliferation, particularly during the transition from $CD4^{-}CD8^{-}$ (DN) to $CD4^{+}CD8^{+}$ (DP) T-cell stages. MicroRNAs likely contribute to abnormal thymocyte maturation and proliferation in T-ALL. While the role of the thymus in advanced T-ALL stages is not fully understood, initial findings suggest its significance in disrupting leukemic cell differentiation. Aberrant Notch signaling affects CXCR4 expression probably modulating immature T-cell migration to the bone marrow, worsening T-ALL progression.

METHODS

In Notch-dependent T-ALL human and murine models, we will explore by FACS analysis, transfection experiments, qRT-PCR, and ChIP assay, the molecular and cellular mechanisms triggered by aberrant Notch signaling, in deregulating microRNAs (miRNAs) expression and CXCR4-dependent T-cell differentiation within the thymus to sustain T-ALL progression. We'll also examine the dysregulated CXCR4-mediated migration of T lymphoblasts between the thymus and bone marrow (BM) niches.

RESULTS

In N3-ICtg compared to WT mice, we detected an anomalous subset of DN thymocytes $CD3\varepsilon^{+}CXCR4^{-}$ displaying altered maturation and migration abilities. Bioinformatic analysis of CXCR4's 3'UTR revealed potential binding sites for specific Notch3-regulated miRNAs enabling leukemic cells to evade differentiative thymus signals and propagate abnormally to the BM.

CONCLUSIONS

In the past decade, numerous studies have explored how microRNAs (miRNAs) influence cancer, particularly in tumor growth and immune evasion. We propose that Notch3, through miRNA modulation, may decrease CXCR4 receptor expression, favoring the survival of an aberrant thymocyte subset that fuels leukemia progression. These findings contribute to a deeper understanding of T-ALL pathogenesis and offer new insights for future therapeutic strategies.

P033

DL922-947 AND SAHA: A COMBINATORY THERAPEUTIC APPROACH AGAINST DIFFERENT AGGRESSIVE CANCERS.

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BACKGROUND-AIM

The oncolytic virus dl922-947 is an adenoviral mutant bearing a 24 bp deletion in the E1A-Conserved Region 2 (CR-2). This mutation allows viral replication only in cells with a non-functional retinoblastoma pathway, a defect observed in the majority of human cancer cells. We have previously demonstrated dl922-947 anticancer effects in several cancer models including malignant mesothelioma, glioma, anaplastic thyroid carcinoma (ATC) and triple negative breast cancer (TNBC).
Novel therapeutic approaches are urgently required for these highly aggressive cancers and with poor prognosis.
In this study, we investigated in ATC and TNBC cell lines a novel combinatory approach using dl922- 947 in combination with suberoylanilide hydroxamic acid (SAHA), an FDA-approved standard histone deacetylase inhibitors (HDAC) inhibitor.

METHODS

We used ATC (8505C, BHT-101) and TNBC (MDA-MB231, MDA-MB468, DU4475) cell lines to evaluate the anti-proliferative effects of dl922-947, SAHA and their combination. Cells were seeded at 500cells/well in 96-well plate and infected with different doses of the virus. SAHA was added at concentrations ranging from 0,05 μM to 5μM. After 6days of incubation, we assessed cytotoxicity of dl922-947, SAHA and their combination by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay and/or sulforhodamine B (SRB) assays. We calculated the IC25 and the IC50 values by GrapPad Prism 9 software. In the combination assays, the virus was used at the IC50 value with different concentrations of SAHA.

RESULTS

We have demonstrated that dl922-947 and SAHA efficiently inhibit ATC and TNBC cell lines proliferation. Furthermore, dl922-947 and SAHA combination potentiated the cytotoxic effect of dl922-947 alone in both ATC and TNBC models.

CONCLUSIONS

These results indicate that dl922-947, particularly when used alongside other drugs like SAHA, may offer a promising and effective therapeutic approach against TNBC and ATC.

P034

NOVEL CIRCULATING BIOMARKERS IN HNSCC PATIENTS RECEIVING ANTI-PD1 THERAPY: THE PREDICTIVE AND PROGNOSTIC ROLE OF IMMUNOSUPPRESSIVE CD137+TREG CELLS AND LOX-1+PMN-MDSCS

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BACKGROUND-AIM

Recurrent/metastatic Head and neck squamous cell carcinoma ((R/M) HNSCC) represents one of the most aggressive and immunosuppressive cancer diseases. Despite the introduction of immunotherapy with Immune checkpoint inhibitors (ICIs), only a limited number of patients obtained long-term benefits. Anti-tumor immune response is in fact defective in these patients, conferring resistance to therapies and promoting tumor progression. Therefore, the identification of novel predictive and prognostic biomarkers giving superior clinical outcomes is still an unmet clinical need in (R/M) HNSCC patients. The impact of circulating immunosuppressive cells on immunotherapy response remains unclear. In this study, we evaluated the role of regulatory T cells (total Treg, resting, active, non-suppressive, and CD137+Treg) and myeloid-derived suppressor cells (MDSCs; LOX-1+PMN-MDSC and M-MDSCs) as possible biomarkers in the blood of (R/M) HNSCC patients underwent immunotherapy.

METHODS

Data from 40 (R/M) HNSCC patients receiving pembrolizumab as first line treatment were prospectively reviewed. PBMCs were isolated at baseline and the levels of Tregs, LOX-1+PMN-MDSC and M-MDSC were analyzed by cytofluorimetry. The percentage of Treg and MDSC cells were correlated with clinical response rate after six months of therapy, progression-free survival (PFS), overall survival (OS) and performance status (PS).

RESULTS

Results showed that responder patients have significantly low levels of circulating LOX-1+PMN-MDSC ($p=0.021$). Moreover, patients with lower levels of LOX-1+PMN-MDSC ($\leq 0.33\%$) and CD137+Treg cells ($\leq 0.081\%$) have prolonged survival ($p=0.03$ and $p=0.01$ respectively). CD137+Treg cells resulted also positively correlated with PS: patients with PS=0 have lower levels of this population than patients with PS>0 ($p=0.01$). Multivariate analysis showed LOX-1+PMN-MDSC cells (>0.33%) as independent prognostic factors correlated with PFS ($p=0.01$) and OS ($p=0.02$).

CONCLUSIONS

Our results indicate that the levels of circulating CD137+Treg and LOX-1+PMN-MDSC cells could predict the success of anti-cancer immunotherapy and could be used as biomarkers for prognosis, monitoring and management of (R/M) HNSCC, improving patient selection and suggesting the development of novel personalized therapeutic strategies

P035

MYC UPSTREAM REGION ORCHESTRATES RESISTANCE TO PI3K INHIBITORS IN COLORECTAL CANCER AND BURKITT LYMPHOMA THROUGH AUTOPHAGIC ADAPTATIONS

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BACKGROUND-AIM

The MYC oncogene is frequently overexpressed in tumors and in particular in most of Colorectal Cancers (CRCs) and Burkitt Lymphomas (BLs). The inhibition of MYC translation in these diseases considered an attractive therapeutic opportunity. An internal ribosome entry site (IRES) was identified in MYC Upstream Region (MYC UR) and was suggested to play a leading role in maintaining elevated MYC content when global cap-dependent translation is inhibited, a mechanism hypothesized to allow the survival of cancer cells during stress and provide resistance to chemotherapy. However, there is conflicting evidence around the validity of MYC IRES translation.
In this study we aim to address if MYC UR can facilitate cell survival in response to various stresses. We also aim to identify a role of MYC UR in chemoresistance to PI3K inhibition and to elucidate the mechanism of action.

METHODS

We utilize a gene editing method to excise MYC UR from Colorectal Cancer cell lines and analyze them combining in vitro and in vivo approaches. We also used Burkitt Lymphoma cell lines either harboring or not a translocated MYC UR along with the MYC open reading frame, depending on the t(8;14) translocation breakpoint.

RESULTS

We found that the genomic deletion of MYC UR neither affected MYC protein content nor CRC cell survival under basal cell culture conditions and in response to stress. We demonstrate that MYC UR does not facilitate cap-independent translation in response to stress, but rather functions as a central enhancer of the autophagic flux in response to PI3K inhibitors. When cells are exposed to PI3K inhibitors, MYC UR facilitates a FOXO3A-dependent transcriptional upregulation of MYC and drug resistance, whereas cells lacking MYC UR are vulnerable and undergo programmed cell death. Mechanistically, this resistance is mediated by an enhancement of the autophagic flux, with MYC as a critical player that promotes the expression of a set of autophagy-related genes. We also demonstrate BL cells lacking the chromosomal translocation of MYC UR succumb to PI3K inhibitors while cells that contain this region translocated respond to PI3K inhibition only if autophagy is blocked.

CONCLUSIONS

The findings challenge previous notions regarding MYC IRES-mediated translation and highlight a promising strategy to overcome resistance to PI3K inhibitors in MYC-driven malignancies. Furthermore, the findings offering potential clinical implications for Colorectal Cancer and Burkitt Lymphoma treatment.

P036

MATCHED GERMLINE AND SOMATIC WHOLE EXOME SEQUENCING: ANALYSIS OF MALE BREAST CANCER PATIENTS WITH MULTIPLE PRIMARY TUMORS.

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BACKGROUND-AIM

Male breast cancer (MBC) is a rare disease often associated with genetic factors. Whole Exome Sequencing (WES) of multiple primary tumors from the same MBC patient may be a powerful tool to elucidate genetic susceptibility to various types of cancer and provide a comprehensive view of shared driver alterations.
The aim of this study was to apply WES for a matched and integrated germline and somatic analysis of high-risk MBC cases with multiple primary tumors, to identify and prioritize candidate pathogenic variants (PVs).

METHODS

We performed WES on 35 germline and tumor DNA samples from a well-characterized series of 13 MBC cases with multiple primary tumors, on Illumina NovaSeq 6000 platform. To identify candidate germline PVs, all PASS variants annotated on open-CRAVAT were filtered to include exonic non-synonymous or splice site variants with 30%≤variant allele frequency (VAF)≤70%, total read depth≥20, and a global allele frequency<1% in the gnomAD database.
The comparison between tumor and matched-normal samples was used to identify true somatic variants. For the detection of driver somatic variants, the filters applied to germline PVs identification were used, with exceptions of 5%≤VAF≤90%, total read depth≥40, variants classified as somatic in the COSMIC database, and variants with a REVEL score≥0.6. COSMIC database was interrogated to determine if the identified driver somatic variants could be clinically actionable.

RESULTS

WES quality was high, showing an average of 96.5% uniformity of coverage for germline and 88.9% for somatic analyses. We identified an average of approximately 37,000 germline and 42,000 somatic PASS variants per sample.
After filtering, eight candidate germline PVs were identified, including PVs in the ATM, BRCA2, ERCC3, and ZFHX3 genes. For somatic variants, an average of 24 candidate driver variants were identified for both MBCs and multiple tumors. According to the COSMIC database, at least one clinically actionable somatic alteration was detected in 4 out of 8 (50.0%) MBCs and 8 out of 13 (61.5%) multiple tumors.

CONCLUSIONS

Results from this study, although further analyses are still ongoing, may contribute to a comprehensive understanding of multi-cancer carcinogenesis, enhancing our understanding of cancer etiology.

P037

EXPRESSION ANALYSIS OF CIRCULATING MICRORNAS FOR THE IDENTIFICATION OF EARLY DIAGNOSTIC BIOMARKERS FOR ORAL SQUAMOUS CELL CARCINOMA

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BACKGROUND-AIM

Oral Squamous Cell Carcinoma (OSCC) accounts for 90% of oral cancers. Its incidence and mortality rates are rising, making it a significant global health challenge. Various risk factors contribute to OSCC development, including lifestyle factors and HPV infection. Identifying molecular biomarkers is crucial as they offer valuable insights for tailoring individualized clinical strategies for OSCC patients. Liquid biopsy (LB) is a minimally invasive procedure used to identify molecular biomarkers, including circulating microRNAs (cmiRNAs).
This study aims to investigate the association of candidate cmiRNAs expression with OSCC onset to identify potential early diagnostic molecular biomarkers.

METHODS

A series of 37 individuals, including 20 patients with OSCC, 12 patients with Potentially Malignant Epithelial Oral Lesions (PMEL), and 5 healthy controls, was enrolled. All participants underwent LB of saliva and plasma samples at the time of enrollment, and RNA was extracted from all samples. For 12 OSCC patients, DNA extracted from FFPE tissue biopsy sections was used to detect high-risk HPV infection using Real-Time PCR. The expression profiles of six cmiRNAs (-21, -31, -138, -145, -184, and -424) in saliva and plasma samples, selected for their roles in OSCC development and progression, were analyzed by Real-Time PCR using miR-16 as the endogenous control. Results were expressed as 2^-ΔCt (Ct target - Ct endogenous control) and compared among the three study groups using the Kruskal-Wallis test.

RESULTS

All OSCC samples tested negative for high-risk HPV infection. Comparing cmiRNA expression levels, cmiR-138 showed a statistically significant decrease in expression in saliva samples from PMEL and OSCC patients compared to healthy controls (p=0.012). No statistically significant differences were found in the expression levels of cmiRNAs in plasma samples among OSCC, PMEL, and controls.

CONCLUSIONS

This study, despite some limitations, provides the first evidence of saliva cmiR-138 as a potential early diagnostic biomarker for OSCC. Saliva LB emerged as a reliable tool for identifying cmiRNAs in OSCC patients, which should be further validated and implemented in clinical settings.

P038

EXPLORING MYELOID-DERIVED SUPPRESSOR CELLS AS TARGET OF IMMUNOCHECKPOINT INHIBITOR THERAPY IN NOTCH3-DEPENDENT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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BACKGROUND-AIM

The role of Immune Checkpoint Inhibitors (ICIs) is being explored in the context of T-cell Acute Lymphoblastic Leukemia (T-ALL). In our T-ALL model, Notch3 transgenic mice (N3tg), tumoral T cells trigger the accumulation of Myeloid-Derived Suppressor Cells (MDSCs). These immature cells inhibit immune responses, thereby promoting tumor progression. The PD-1/PD-L1 pathway is essential for T-cell activation and proliferation. MDSCs contribute to tumor growth through various mechanisms, including the expression of PD-L1, which inhibits PD-1^+ cells within the tumor microenvironment, such as T- and NK-cells. Our goal is to explore MDSCs as a potential therapeutic target for T-ALL, aiming to reactivate NK immune responses, by modulating the PD-1/PD-L1 axis. Additionally, we seek to investigate the possible side effects of ICI treatment.

METHODS

We administrated both a single anti PD-L1 and a combined anti-Gr-1/anti-PD-L1 therapy to N3tg mice. The treatment was performed, via intraperitoneal injections, as follows: 200 μg/mouse, and 100 μg/mouse, twice a week for 2 weeks for anti Gr-1 and anti PD-L1, respectively. At the end of the treatment, N3tg mice and relative untreated controls, were sacrificed and appropriate spleen subsets were isolated to evaluate suppressive function of MDSCs and NK cytotoxic activity.

RESULTS

Our data demonstrate that during T-ALL progression, there is an accumulation of both PD-$L1^+$ MDSCs and PD-1^+ NK cell subsets, corresponding to about 30% of total cells. In response to these findings, we administrated anti-PD-L1 antibody treatment, resulting in a reduction in tumor cells and MDSCs, accompained by an increase in NK PD-1^+ cells and an enhancement of their cytotoxic function. Moreover, our preliminary results indicate that the anti-Gr-1/anti-PD-L1 combined therapy, to hit both differentiation and function of MDSCs, respectively, improves inhibition of disease progression. Interestingly, in about 10% of anti PD-L1 treated mice, we also observed severe side effects reminiscent of ICI-associated type 1 diabetes.

CONCLUSIONS

Our aim is to further explore, in Notch3-dependent T-ALL, the efficacy of the combined therapy that targets MDSCs, and to deeper investigate molecular mechanism/s regulating possible side effects of ICI-based therapy.

P039

UNVEILING TRIM8 BY EXPLORING ITS ROLE AS A MEDIATOR OF INNATE IMMUNE SIGNALING MODULATION IN INFLAMMATORY CONDITIONS: A SYSTEMATIC REVIEW

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BACKGROUND-AIM

Tripartite motif-containing protein 8 (TRIM8) is a RING finger protein belonging to the TRIM protein family, one of the largest families of RING domain-containing E3 ligases that mediate post-translational modifications of proteins. TRIM8 dysfunction is linked to cancer, inflammatory processes and autoimmune disorders, due to its involvement in the regulation of three pivotal cellular signaling pathways: the p53 tumor suppressor signaling pathway, the NF-κB pathway and STAT3 (Signal Transducer and Activator of Transcription 3) of the JAK-STAT pathway. Here, we dissected about the pivotal role of TRIM8 as mediator of the inflammatory pathways.

METHODS

This review follows the PRISMA Statement (Preferred Reporting Items) guidelines for systematic reviews and meta-analyses) for scoping reviews. The literature search was carried out on 02/21/2024 in the National Library databases of Medicine MEDLINE/PubMed and Scopus.

RESULTS

Overall, 43 articles were retrieved from the initial search. After removing 17 duplicates, a total of 26 reports were screened for title and abstract in order to eliminate all the not relevant articles. After a careful and accurate reading, 11 articles were included in this review.

CONCLUSIONS

TRIM8 has a pivotal role in inflammation since interact with different proteins involved in different inflammatory pathways: it is a negative regulator of innate immune and inflammatory responses mediated by Toll-Like Receptors 3 and 4 (TLR3 and TLR4); TRIM8 activates the NF-κB signaling pathway acting both in the cytoplasm and in the nucleus and specifically interacts with SOCS-1 (Suppressor Of Cytokine Signaling-1), leading to its degradation and allowing the activation of JAK-STAT induced by IFNγ.

The role of TRIM8 in inflammatory pathways has so far been investigated in several pathologies, including acute lung injury (ALI), Osteoarthritis (OA), Ulcerative colitis (UC), Hepatic ischemic reperfusion (I/R), ischemic reperfusion-mediated cerebral injury and in contrast-media-induced nephropathy (CIN), in which TRIM8 has been recognized as a potential target for pharmaceutical agents due to its role in the inflammation pathways.

P040

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 acts as an oncosuppressor by modulating FGF/FGFR signaling and inflammation. 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. The activation of FGF/FGFR system was investigated through western blot analysis. 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 in vitro, and that both treatment with recombinant PTX3 and its endogenous/genetic overexpression reduce LEC activation by inhibiting FGF/FGFR signaling. Furthermore, we observed that in a transgenic mouse model, lymphatic expression of PTX3 hampers lymphangiogenesis and significantly reduces the metastatic spreading of melanoma to the draining LN in two different cell dissemination models.

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.

P041

THE MEK5/ERK5 PATHWAY PROMOTES THE ACTIVATION OF THE HEDGEHOG/GLI SIGNALLING IN MELANOMA CELLS

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BACKGROUND-AIM

Malignant melanoma is among the most aggressive cancers and its incidence is increasing worldwide. We previously reported that both the Hedgehog/GLI (HH/GLI) signalling and the Mitogen-Activated Protein kinase ERK5 promote melanoma growth, and that GLI1 regulates ERK5 activation and that the latter is required for Hedgehog/GLI (HH/GLI)-dependent melanoma cell proliferation. Since the HH/GLI pathway may be activated in a non-canonical way by the MAPK ERK1/2, we explored whether ERK5 regulates the HH/GLI signalling.

METHODS

BRAFV600E-mutated (A375 and SK-Mel-5) and wild-type BRAF (SSM2c) melanoma cell lines and murine NIH/3T3 fibroblasts were silenced for ERK5 using ERK5-targeting shRNAs or treated with a non-targeting shRNA (shNT) as a negative control. Luciferase assay using the GLI-binding site luciferase reporter was performed to evaluate GLI transcriptional activity. A constitutively active form of MEK5 (MEK5DD) was used to induce activation of endogenous ERK5 or overexpressed ERK5. Chemicals (small molecule inhibitors) used were: the ERK5 inhibitors JWG-071 and AX15836; MEK5 inhibitors GW284543 and BIX02189; GLI1/2 inhibitor GANT61; SAG, an HH/GLI pathway activator. Activation of HH/GLI pathway was obtained by PATCH1 silencing. 3D speroid assays were performed in SSM2c and A375 cells treated with GANT61 in combination with the MEK5 inhibitors.

RESULTS

Genetic and pharmacological inhibition of ERK5 reduces GLI1 and GLI2 protein levels and transcriptional activity of endogenous HH/GLI pathway induced by the agonist SAG in NIH/3T3 cells. In these cells, ERK5 activation by overexpression of a constitutively active MEK5 mutant (MEK5DD) potentiates the transcriptional activity of endogenous HH/GLI pathway induced by SAG, whereas ERK5 genetic inhibition prevents this effect. Consistently, MEK5DD overexpression increases GLI1 and GLI2 protein levels. In melanoma cells, ERK5 knockdown reduces mRNA and protein expression levels of GLI1 and GLI2 and inhibits GLI transcriptional activity. MEK5DD further increases the transcriptional activity of the HH/GLI pathway and GLI protein levels. Interestingly, the combination of GLI and MEK5 inhibitors is more effective than single treatments in reducing melanoma spheroid growth.

CONCLUSIONS

Combined targeting of the MEK5/ERK5 and HH/GLI pathways may be a useful approach to prevent resistance mechanisms frequently observed upon monotherapy in melanoma.

P042

PRESENCE OF RAFT COMPONENTS WITHIN EXTRACELLULAR VESICLES SECRETED BY HUMAN FIBROSARCOMA CELLS FOLLOWING AUTOPHAGY TRIGGERING

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BACKGROUND-AIM

Autophagy plays a crucial role in selectively eliminating protein aggregates and dysfunctional organelles, with the molecular constituents from this degradation, such as amino acids, lipids, and sugars, supporting cell survival, particularly under nutrient deprivation. Our preliminary observations indicated that autophagy induces the formation of a subset of large heterogeneous intracellular vesicular structures. In this study, we examined extracellular vesicles (EVs) induced by autophagy in human fibrosarcoma 2FTGH cells, offering insights into the interplay between autophagy and EVs may enhance our knowledge of disease mechanisms and reveal novel therapeutic targets for diseases.

METHODS

We analyzed by morphological and biochemical approaches EVs induced by autophagy in human fibrosarcoma 2FTGH cells. A self-forming iodixanol gradient was used for cell subfractionation, followed by Western blot analysis to determine the co-fractionation of LC3-II with CD63 and CD81. Immunogold electron microscopy and coimmunoprecipitation were employed to analyze the enrichment of raft components (GD3, ERLIN1) within EVs.

RESULTS

EV characterization revealed that autophagy-induced cells release both plasma membrane-derived microvesicles and exosomes. Western blot analysis demonstrated that LC3-II co-fractionated with CD63 and CD81. Further analysis indicated that raft markers GD3 and ERLIN1 co-fractionated with LC3-II. Dual staining by immunogold electron microscopy and coimmunoprecipitation revealed GD3-LC3-II association, indicating that autophagy promotes enrichment of raft components within EVs.

CONCLUSIONS

Our findings introduce a new aspect of the interaction between autophagy and the endolysosomal system, highlighting the role of autophagy in the enrichment of raft components within EVs. This insight could have significant implications for understanding pathogenic mechanisms and developing alternative raft-targeted therapies in diseases where EV generation is prevalent.

P043

STRUCTURE-BASED VIRTUAL SCREENING AND FUNCTIONAL VALIDATION OF POTENTIAL INHIBITORS TARGETING HUMAN LACTATE DEHYDROGENASE

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BACKGROUND-AIM

In comparison with normal cells, most cancer cells rely on aerobic glycolysis to obtain energy and necessary glycolytic intermediates which can be used for protein synthesis and cell division. It results in much higher glucose uptake by cancer cells compared to normal ones, and glucose conversion into lactate regardless to the presence of extracellular oxygen. Since this aerobic conversion of pyruvate into lactate is catalyzed by lactate dehydrogenase (LDH), we searched for novel inhibitors of this enzyme, able to decrease growth advantages associated with cancer cells. As a study model, we used medulloblastoma (MB), a tumor characterized by a reprogramming of energy metabolism toward aerobic glycolysis.

METHODS

Through structure-based virtual screenings, we identified potential LDHA inhibitors and we tested them by proliferation assays. Cells were incubated for 24h with compounds and then counted by Trypan Blue exclusion method. To test their specificity, we generated an inducible LDHA-deficient cell line, using a lentiviral vector expressing shRNA targeting LDHA mRNA, under the control of a doxycycline-inducible promoter. Cells were stably transduced with the lentiviral vector, the knockdown was induced with doxycycline for 24h and following cell proliferation was evaluated. Specificity was confirmed by LDHA enzymatic assay, where the inhibition of LDH was evaluated by measuring the rate of NADH consumption by spectrophotometry. For Western blotting analysis cells were treated, collected, lysed and proteins were visualized by enhanced chemiluminescence.

RESULTS

We identified a novel and specific LDH inhibitor, compound #18, which showed a robust anticancer activity. To test its specificity, we performed enzyme assays upon #18 treatment and we observed a significant inhibitory effect on LDHA activity. To determine if the anticancer activity was due to death or autophagic mechanisms, we analyzed PARP cleavage and LC3B I/II levels by western blot, that did not show significant changes. On the contrary, when cells were exposed to #18 and Rotenone, a complex I inhibitor, proliferation was strongly decreased and programmed cell death was induced.

CONCLUSIONS

Our results support the conclusion that #18 is an effective, potent and specific LDHA inhibitor which deserves to be further investigated as a starting point for the development of novel anticancer strategies based on the targeting of tumor-specific key metabolic steps.

P044

PRE-CLINICAL EVIDENCE OF CDK4/6 INHIBITION AS A POTENTIAL THERAPEUTIC STRATEGY IN EWING SARCOMA

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BACKGROUND-AIM

Ewing sarcoma (EWS) is a rare and aggressive tumor of bone and soft tissue that occurs prevalently in children and adolescents. Treatment options, such as surgery, radiation, and chemotherapy, have improved patient outcomes; however, metastatic disease occurs within the first few years after treatment. EWS is characterized by a chromosomal translocation, which results in the fusion gene EWS/FLI1. The oncogenic protein EWS/FLI1 contributes to tumorigenesis by modulating cell cycle regulators, including cyclin D1. Moreover, cyclin D1 and CDK4 are highly expressed in EWS. Therefore, in this study we evaluated the effects of CDK4/6 inhibitors in EWS cells and investigate their potential to improve the efficacy of standard chemotherapy.

METHODS

The study was performed in a panel of EWS cell lines. Cell cycle distribution, cell proliferation (2D and 3D), cell death, colony formation, modulation of survival/proliferation signaling pathways, and cell energy metabolism were evaluated in cells treated with CDK4/6 inhibitors alone or in combination with chemotherapy.

RESULTS

Among the CDK4/6 inhibitors tested, abemaciclib was the most effective in inhibiting cell proliferation. Abemaciclib inhibited RB phosphorylation, downregulating the expression of the E2F target myc. In contrast with palbociclib, abemaciclib induced an irreversible G1 cell cycle arrest, which resulted in the failure to restore cell proliferation after drug withdrawal. These effects were presumably associated with the ability of abemaciclib to inhibit CDK9 in addition to CDK4/6. Interestingly, abemaciclib reduced glucose utilization, downregulating AKT-dependent GLUT-1 glucose transporter expression. Then, we studied the effects of abemaciclib combined with etoposide, doxorubicin or vincristine, following simultaneous or combined treatment schedules. Simultaneous treatment with abemaciclib and vincristine had proven to be the most effective in enhancing the growth-inhibitory effects of single agents either in 2D or 3D culture. In addition, this combination inhibited colony formation and induced cell death more strongly than single agents.

CONCLUSIONS

Our pre-clinical results proved the therapeutic potential of the CDK4/6 inhibitor abemaciclib for the treatment of EWS, alone or combined with vincristine-based chemotherapy.

P045

ASGR2 AND CLEC12A C TYPE LECTINS: NEW PLAYERS IN GLIOBLASTOMA IMMUNOSUPPRESSIVE NETWORKS

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BACKGROUND-AIM

Glioblastoma (GBM) is the most fathal brain tumor in adults with poor survival and no effective treatment. Dissecting immunosuppressive mechanisms in GBM is crucial to identify novel therapeutic targets. Lectins are glycan-binding immune receptors, that promote immunosuppression in cancer. Little is known about lectin immune receptors (C-type lectins, Siglecs, galectins) and their role in GBM. Here, we aim to identify novel lectins that could be relevant in GBM immunosuppression.

METHODS

Differential network analysis was performed using publicly available databases (TCGA and CGGA for IDH-WT GBM; GTEx for normal brain) to identify lectins positively coordinated in GBM. Based on the lectins' expression, patients' subgroups were identified using Fuzzy C-means algorithm and survival analysis was performed. To validate the identified lectin expression profile on immune cell subsets, patients-derived tumor samples (10) were freshly processed to obtain a single cell suspension and analyzed by flow cytometry.

RESULTS

Differential co-expression network between TCGA and GTEx identified a community of lectins, whose coordination was increased in GBM. ASGR2 and CLEC12A emerged as the strongest hubs in the network. To validate our finding, we performed the differential network analysis on CGGA, that confirmed ASGR2 and CLEC12A as the strongest hubs. Up today, no evidences are available on ASGR2 and CLEC12A role in GBM, so we characterized for the first time their expression on immune cells in patients-derived GBM tumors.
We found that intratumoral CD45+ cells express both ASGR2 and CLEC12A, while CD45- cells are negative. ASGR2 is predominantly expressed by infiltrating macrophages than resident microglia ($p<0.05$). CLEC12A is associated to both infiltrating and resident immune cells. Stratifying patients based on ASGR2 and CLEC12A expression, we found that high levels of ASGR2-CLEC12A correlated with the worst prognosis ($p<0.05$).

CONCLUSIONS

Our results show for the first time the expression of ASGR2 and CLEC12A exclusively on immune infiltrate in GBM. These lectins belong to C-type lectin family, that has been poorly investigated in GBM. Given their association with immune infiltrating cells, ASGR2 and CLEC12A could be relevant and contribute to GBM immunosuppression.

P046

THE LYSINE METHYLTRANSFERASE SETD8 REGULATES AUTOPHAGIC FLUX AND SENSITIVITY TO TEMOZOLOMIDE IN GLIOBLASTOMA CELLS

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BACKGROUND-AIM

Despite intense research efforts, glioblastoma (GB) remains an incurable brain tumor. In searching for more effective drug targets, we have recently found that the H4K20 methyltransferase SETD8 was overexpressed in 50% of the gliomas and that the SETD8 inhibitor UNC0379 restrained GB proliferation in cellular and mouse models. In performing these experiments, we noticed that UNC0379 also caused a dramatic accumulation of aberrant vesicles in GB cell cytoplasm. Thus, we set up to investigate the nature of such vesicles.

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

The UNC0379-induced aberrant vesicles were positive for the lipidated form of LC3B, suggesting their autophagic nature. Western blot experiments showed a strong accumulation of both lipidated LC3B and p62 (SQSTM1), strongly suggesting a block in the autophagic flux. As SETD8-mediated H4K20 methylation regulates transcription, we investigated the effects of UNC0379-induced SETD8 inhibition on the transcription of several genes coding for master regulators of autophagy. We found that UNC0379 treatment induced transcription of p62. Accordingly, we found, by ChIP and ChIP-Seq, that SETD8-induced H4K20 methylation resulted in p62 promoter repression. As autophagy inhibition sensitizes tumor cells to DNA-damaging drugs we, thus, rationalized to combine UNC0379 with the GB drug of choice Temozolomide, a DNA damaging agent. Indeed, the combination UNC0379+Temozolomide strongly induced apoptosis in GB cells.

CONCLUSIONS

SETD8 regulates autophagic flux by tuning p62 transcription. UNC0379-induced SETD8 inhibition resulted in aberrant p62 transcription and in autophagic flux block. This mechanism has been successfully exploited to sensitize GB cells to the DNA damaging effects of temozolomide, providing a strong rationale to further test UNC0379+temozolomide therapeutic efficacy in orthotopic mouse models.

P047

CIRCULATING MIRNAS IN IDIOPATHIC PULMONARY FIBROSIS: A PILOT STUDY.

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BACKGROUND-AIM

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fibrotic disease characterized by exertional dyspnea and non-productive chronic cough. Although the pathogenesis of IPF is unknown, genetic and environmental factors could be implicated in the development of the fibrotic process. Currently, early and/or minimally invasive biomarkers are not available for this disease. In this context, liquid biopsy techniques are gaining an increasingly important role in the evaluation of such biomarkers. Particularly, circulating microRNAs (miRNAs) can provide valuable insights into the underlying pathogenetic mechanisms of complex and multifactorial diseases. In this study, we aimed at investigating the profile of circulating miRNAs in patients with IPF.

METHODS

We analised the profile of circulating miRNAs through small RNA sequencing (RNASeq) in the plasma of 14 patients with IPF and 7 healthy subjects. All patients aged between 59 and 88 years, and had high-resolution CT features suggestive for definite or probable usual interstitial pneumonia (UIP).

RESULTS

Thirty differentially expressed miRNAs were identified, including 21 up-regulated and 9 down-regulated. This profile was found to be unique for this type of pathology, showing no overlap with circulating miRNAs identified in other fibrotic diseases such as liver cirrhosis and autoinflammatory diseases such as Systemic Lupus Erythematosus and vasculitis like Behçet's syndrome. Functional analysis using Gene Ontology (GO) revealed several terms related to fibrosis, particularly signaling mechanisms mediated by GTPases and cell adhesion molecules. Analysis conducted through the KEGG ontology showed significant enrichment for the term Non-Small Cell Lung Cancer (NSCLC). Specifically, among the target genes and miRNAs deregulated in patients with IPF, we identified genes relevant to the pathophysiology of this type of tumor (ALK, EGFR numerous kinases, genes related to apoptosis, etc.).

CONCLUSIONS

Overall, the results of the present study, albeit preliminary, open up interesting views for studying pathophysiological mechanisms and lay the groundwork for the use of miRNAs as early biomarkers of IPF and its progression.

P048

TARGETING POLYAMINE METABOLISM/EIF5A-HYPUSINATION AXIS FOR FAP AND COLORECTAL CANCER THERAPY

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BACKGROUND-AIM

Colorectal cancer (CRC) is the third-most lethal cancer worldwide. Pathways altered in CRC lead to activation of the MYC oncogene, which is not targetable directly due to its flat protein structure. It is possible to inhibit processes regulated by MYC function, such as polyamine metabolism. Polyamines are small polycations involved in many cellular functions, increased in different cancer types included CRC. Among them, spermidine is involved in the post-translational modification of eIF5A, a translation factor able to alleviates ribosome stalling at specific pausing motifs present in selective transcripts. Polyamine production is mostly regulated by the ornithine decarboxylase (ODC) a first-rate limiting enzyme. The ODC inhibitor DFMO efficiently prevent tumor growth in different settings, leading to clinical trials in CRC patients. The therapeutic benefit of this approach was limited, because of resistance due to the upregulation of polyamine transporters and increased polyamine uptake.The aim of our study is to unveil more efficient therapeutic strategies and new molecular targets to overcome problems due to resistance

METHODS

Molecular and cellular biology tecniques, in vivo approaches using xenograft models in nude mice, and APCmin mice for F.A.P. and CRC. Bioinformatic analysis and pharmacological studies to explore the potential of the drugs analyzed, alone or in combination

RESULTS

We studied the effects of polyamines/eIF5A axis inhibition, by using a polyamine-synthesis inhibitor GC7 alone or in combination with DFMO in order to overcome chemoresistance. We found that MYC is a key translational target of hyp-eIF5A, the direct inhibition eIF5A-mediated elongation of MYC efficiently suppresses FAP and intestinal tumorigenesis in mice. We demonstrated that combined inhibition of ODC and hyp-eIF5A induces a synergistic antitumor response in CRC cells, leading to complete suppression of MYC translation by preventing translational elongation and initiation. We found that genes of the polyamine/hypusine biosynthesis are significantly upregulated in CRC patients and that the blockade of ODC or hypusination alone limits CRC proliferation through a cytostatic mechanism, while their combined inhibition induces a synergistic inhibition, together with apoptotic cell death in vitro and in mouse models of CRC and FAP

CONCLUSIONS

Our data depict a novel therapeutic strategy for FAP and CRC, based on the combined suppression of ODC and hyp-eIF5A which hold promise for future patient treatments

P049

NANO-SIZED VESICLES FROM BRASSICA OLERACEA L. (BROCCOLI) PROMOTES PHAGOCYTOSIS OF APOPTOTIC NEUTROPHILS BY MACROPHAGES

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BACKGROUND-AIM

The clearance of apoptotic cells by macrophages — a process termed 'efferocytosis' — is essential for the resolution of inflammation. Accordingly, defective efferocytosis underlies several inflammatory diseases. In recent years, natural plant-derived products have received increasing attention since they might have a role in the modulation of macrophage activation, recruitment, and polarization, as well as in the metabolic status of macrophages. Our work highlights the potential application of Broccoli Derived Nanovesicles (BDNVs) to target macrophages so as to improve their efferocytic capacity. We hypothesized that apoptotic neutrophils removal by macrophages could be improved by their pretreatment with different doses of BDNVs.

METHODS

Differentiation of monocytic THP-1 cells in adherent macrophages was achieved by addition of phorbol myristate acetate (PMA) for 72 h. Human neutrophils were isolated using Ficoll density gradient and their apoptosis was achieved by thermal shock at 43°C for 60 min followed by an incubation at 37°C for 24 h. For isolation of BDNVs, fresh sprouts of Brassica oleracea L. (Broccoli) were subjected to ultracentrifugation and their amount were expressed as weight/volume. PKH-26 labeled apoptotic neutrophils were fed to BDNVs-treated macrophages and phagocytic activity was analyzed by epifluorescence microscopy and flow cytometry.

RESULTS

Macrophage morphology was confirmed by microscopic observation while neutrophil apoptosis was assessed by trypan blue-exclusion method. Interestingly, our data showed that when pre-treated with BDNVs, macrophages exhibited a significantly increased phagocytic competence for apoptotic neutrophils as compared with untreated macrophages. In particular, epifluorescence showed a heightened intracellular PHK-26 signal depending on BDNV concentration (from 5 to 100 μg/mL). These data were confirmed by flow cytometric analysis which detected an increase in the percentage of PHK-26 positive macrophages when treated with BDNVs (in a wide range of 0-5000 μg/mL).

CONCLUSIONS

Taken together, these data showed that BDNVs could act as pro-resolvent agent that triggers the prompt clearance of apoptotic neutrophils by enhanced efferocytosis activity.

P050

IL-1 FAMILY CYTOKINES AND SOLUBLE RECEPTORS IN HIDRADENITIS SUPPURATIVA

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BACKGROUND-AIM

Hidradenitis suppurativa (HS) is a chronic skin disease, characterized by clinical inflammation of the hair follicle with the recurrence of abscesses, nodules, and tunnels.
Recently, several studies suggested a role of IL-1 family (IL-1F) cytokines in eliciting and sustaining the disease.
The aim of this work is to perform a comprehensive analysis of IL-1F cytokines, soluble inhibitors and receptors in a cohort of HS patients not treated with biological agents

METHODS

Sixteen patients affected by HS and 16 healthy controls were recruited; clinical data were collected and disease severity evaluated by means of the International HS Severity Score System (IHS4). Serum levels of IL-1F cytokines, inhibitors and receptors were measured using a Multiplex Assays.

RESULTS

IL-18, free IL-18 and IL-17A levels were significantly higher in patients vs controls. Among soluble inhibitors, IL-1Ra, IL-1R2 and ST2/IL-1R4 were significantly increased. IL-18, free IL-18 and IL-33 levels are strongly correlated with IHS4. Also the inhibitors IL-1Ra and IL-18BP show a correlation with IHS4

CONCLUSIONS

The data obtained in this study confirm the involvement of IL-1F cytokines in mediating the disease and determining its severity and suggest a possible role for IL-18 as novel serum biomarker of active disease.

P051

MAML1 OVER-EXPRESSION PROMOTES TRIPLE-NEGATIVE BREAST CANCER VIA NOTCH1 AND GLI1 BY INDUCING ITCH/E3 LIGASE SELF-UBIQUITYLATION

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BACKGROUND-AIM

Mastermind-like-1 is a known many-sided transcriptional cofactor, capable of crosstalk between major signaling pathways, including Notch and Hedgehog. Maml1 has been recently shown to act as a coactivator in other signalling pathways, such as p53, Wnt and Hippo in a Notch-independent manner. Hedgehog and Notch signalling pathways are directly involved in the onset/development of several cancers and are both regulated at the post-translational level by Itch/E3 ubiquitin ligase protein. Beyond the co-transcriptional function, we first show that Maml1 overexpression can control the post-translational modifications of ubiquitin signaling on Itch-targeted substrates, as Notch1 and Gli1, working as 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

We report a functional interaction between the Maml1-PPQY motif and Itch-WW domains, leading to K63-linked Itch auto-ubiquitylation with a degradative effect, Maml1-induced. Conversely, Itch is protected by poly-ubiquitination events in the $Mam1^{-/-}$ mouse model, resulting in high protein stability. Hence, we demonstrate that enforced expression of Maml1 can switch off Itch expression/activity resulting in a strong up-regulation of Notch1 and Gli1, favoring their oncogenic activity. Accordingly, in Triple Negative Breast Cancer, Maml1 up-regulation drives accelerated tumor growth and faster distant multiorgan metastasis in vivo, strongly suggesting an oncogenic function for Maml1.

CONCLUSIONS

Our data demonstrate a dual role for Maml1 overexpression, acting as transcriptional co-factor of major pathways, and post-translational regulator of Itch target proteins, impinging on Itch expression, ultimately destroying several signaling pathways underlying TNBC heterogeneity. The characterization of Maml1 as a novel negative regulator of Itch adds a piece in the understanding of tumour biology by identifying Maml1 as a potent tumor driver that may become an attractive therapeutic target for TNBC.

P052

GLUTAMINE AND ASPARAGINE DEPENDENCE OF BONE-METASTATIC PROSTATE CARCINOMA CELLS UNDER PHYSIOLOGICAL CULTURE CONDITIONS

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BACKGROUND-AIM

During tumor progression, prostate carcinoma (PCa) is associated with marked metabolic changes. In particular, in a number of in vitro studies metastatic PCa cells exhibit a strong dependence on glutamine (Gln). However, results obtained in pre-clinical models and, more importantly, in clinical trials with glutaminase inhibitors or glutamine analogs have been generally disappointing. Here, we have studied Gln metabolism of PCa cells in vitro under conditions mimicking physiological bone marrow environment, which is the main metastatic site of PCa cells.

METHODS

Two human metastatic PCa cell lines (PC-3 and VCAP) were cultured in conventional (DMEM, asparagine absent) or physiological plasma-like (Plasmax, asparagine present) medium in the absence or in the presence of Gln. PCa cells were also seeded in the top compartment of a double-chamber co-culture system, with bone marrow mesenchymal stromal cells (MSCs) placed in the bottom chamber. Cell viability and the expression of Gln-associated genes and proteins were evaluated in cancer cells.

RESULTS

Both PCa cell lines showed a dramatic loss of viability when incubated in the absence of Gln in DMEM, although cells expressed the enzyme Glutamine Synthetase and up-regulated the protein upon Gln deprivation. Asparagine (Asn) and a membrane-permeant analogue of 2-oxoglutarate significantly and additively rescued cell viability of Gln starved PC-3 cells, while viability rescue was almost completely attributable to Asn in VCAP cells, although both cell lines express the enzyme Asparagine Synthetase. In contrast, Gln depletion only slightly lowered cell viability of both PCa cell lines in Plasmax medium. However, even in this medium cell viability was markedly hindered by L-Asparaginase, which hydrolyses both Gln and Asn. Finally, in both Gln-depleted DMEM and Plasmax, co-cultured MSCs exerted a partial, although significant, protection on PCa cells.

CONCLUSIONS

These preliminary results suggest that Asn availability is strictly required by PCa cells, while their Gln-dependence is exaggerated in not physiological, conventional media. Moreover, PCa cells may arrange a metabolic interaction with bone marrow MSCs, which protects cancer viability under nutritional stress.

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P053

CORRELATION BETWEEN FAECAL CALPROTECTIN LEVELS AND GUT MICROBIOME PROFILE IN HEALTHY SUBJECTS FROM NORTHERN ITALY

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BACKGROUND-AIM

The main biomarker used to diagnose and monitor Inflammatory Bowel Disease (IBD) is faecal calprotectin (FC), which is mainly released by recruited neutrophils, thus reflecting local inflammatory status. However, several confounding factors are associated with high levels of FC, such as other gastrointestinal diseases, drugs, lifestyle, and age, making FC a sensitive but low-specificity IBD marker. Gut microbiota composition is altered in IBD patients and could represent an additional, non-invasive biomarker. Here, we have evaluated if a correlation exists between microbiota composition and FC levels in subjects with no known pathologies in a specific area of northern Italy, considering several possible factors that might influence FC levels.

METHODS

500 subjects (age 18-85 yrs, 60% females and 40% males), resident in the Parma area (Emilia-Romagna, Italy), were enrolled in the context of a larger project (the Parma Microbiota study EC 1108/2020/TESS/UNIPR), and their potentially confounding factors were obtained through questionnaires. Faecal samples were collected, and 15mg of each sample were picked up with specific devices to quantify FC levels through ELISA analysis. Bacterial DNA was extracted and purified from the same faecal samples and next-generation shallow shotgun metagenomics analysis was performed through an Illumina sequencing platform.

RESULTS

Median FC in the studied population was 23.6 μg/g. More than 70% of donors showed FC levels below the cut-off considered normal (50 μg/g), while about 5% of subjects presented FC levels higher than 150 μg/g, potentially indicating a high inflammatory status. High FC levels have been associated with the presence of several opportunistic pathogen bacteria, such as Escherichia coli and Klebsiella variicola, while low FC levels correlated with the presence of butyrate-producing species, belonging to Butyrivibrio and Roseburia genera.

CONCLUSIONS

In conclusion, 5% of a northern Italy adult population with no known pathologies display FC levels indicative of an intestinal inflammatory condition, suggesting a possible predisposition to develop the IBD. If confirmed in a larger population, the relationship between FC levels and microbiota composition will allow to identify microbial biomarkers associated with a higher risk of IBD.

Acknowledgement: Giuseppe Taurino is supported by Fondazione Umberto Veronesi, Italy.

P054

IDENTIFICATION OF A RELATIONSHIP BETWEEN ERK5 AND KARYOPHERIN α FAMILY IN MELANOMA

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BACKGROUND-AIM

The Mitogen-Activated Protein Kinase (MAPK) Extracellular-signal Regulated Kinase 5 (ERK5) is emerging as a possible target for cancer treatment. ERK5-dependent sustainment of cancer proliferation is linked to its presence in the nucleus, but the nuclear transporters involved in ERK5 cyto-to-nucleus shuttling have not been fully identified. ERK5 has an N-terminal kinase domain and a long C-terminal tail containing a transcriptional transactivation domain and a classical nuclear localization signal (cNLS), and possesses a high molecular weight so that it needs to be actively transported into the nucleus. Many of the nuclear transporters belong to the karyopherin β (or importin β) and karyopherin α (or importin α) superfamily. In addition, it is known that cNLS on cargo proteins are recognized by importin α that, in turn, heterodimerizes with the β1 subunit. Importin β1, in turn, interacts with the nucleoporins on the nuclear pore complex, allowing protein nuclear translocation. The presence of a cNLS in ERK5 structure suggested the involvement of α/β1 importins, and we recently showed that importin β1 mediates ERK5 nuclear translocation, and that its inhibition synergizes with ERK5 kinase inhibitors in reducing cancer cell proliferation.

METHODS

To shed light on the possible importin α involved in ERK5 nuclear shuttling, we used the TCGA
publicly available melanoma dataset on cBioportal and TNMPlot. Moreover, we used Importazole (IPZ), an inhibitor of the α/β1-mediated import.

RESULTS

On cBioportal we found that among the 7 human karyopherin α (KPNA) components, KPNA2 mRNA levels positively correlated with those of the ERK5 target genes KLF2, MEF2D and MYC. Interestingly, using TNMPlot we found that KPNA2 expression was significantly higher than in normal tissues in several types of cancers, among which skin cancer. In particular, in skin cutaneous melanoma, the expression of KPNA2 was higher in both primary and metastatic tumours with respect to normal tissues. In addition, KPNB1 (karyopherin β1) expression was higher in skin cancer and also in primary and metastatic skin cutaneous melanoma compared to normal tissues. Importazole (IPZ) reduced the nuclear amount of ERK5 and synergized with ERK5 kinase inhibitors in reducing the proliferation of A375 melanoma cells.

CONCLUSIONS

In conclusion, identification of the karyopherin α involved in ERK5 nuclear shuttling is crucial to develop new drugs that alone, or in combination with ERK5 kinase activity inhibitors, may reduce tumour growth.

P055

EVIDENCE OF NEUROGLOBIN AS A POSITIVE REGULATOR OF AUTOPHAGY IN HUMAN NEUROBLASTOMA CELLS

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BACKGROUND-AIM

Neuroglobin (Ngb) is a hexacoordinated hemeprotein mainly expressed in neurons. Following its upregulation and mitochondrial localization, Ngb appears to play a pro-survival role against oxidative stress. However, the molecular mechanisms underlying its action are only partly known. Since autophagy is a highly conserved catabolic process which preserves cell homeostasis in stress conditions, we analysed both the proteome and the interactome of Ngb-overexpressing neuroblastoma cells to investigate whether, and how, Ngb upregulation could promote autophagy.

METHODS

Liquid chromatography-tandem mass spectrometry, label-free quantification and bioinformatic enrichment analysis were performed to characterize the proteome of stably transfected Ngb-overexpressing SH-SY5Y cells (NGB-FLAG) compared to negative control (CTRL). LC3-II, p62 and LAMP1 levels were analysed by western blot to globally evaluate autophagy. Ngb autophagosome localization was determined by anti-LC3-II immunoprecipitation experiments (IP). Finally, autophagy-related Ngb interactors were identified by affinity purification-mass spectrometry and protein-protein interaction (PPI) network analysis and validated by IP.

RESULTS

Proteome analysis identified 107 significantly upregulated proteins in NGB-FLAG compared to CTRL, among which LAMP1 increment was validated by western blot analysis. The significant increase of LC3-II and decrease of p62 levels in NGB-FLAG compared to CTRL revealed that Ngb overexpression induces autophagy. Besides, the concomitant detection of both Ngb and LAMP1 in anti-LC3-II IP samples demonstrated the presence of Ngb in autophagolysosomes. Finally, PPI network analysis indicated Raptor (Regulatory-associated protein of mTOR) as a putative Ngb-binding partner, which was then validated by the detection of Ngb in anti-Raptor IP samples, at significantly higher levels in NGB-FLAG than CTRL.

CONCLUSIONS

Ngb may have unique functions other than oxygen supply. Collectively, our data strongly suggest that Ngb could be a positive regulator of autophagy, involved in both early and late autophagic machinery. Further experiments may elucidate whether autophagy stimulation might be a mechanism through which Ngb could prevent cell death.

P056

GOLD NANOPARTICLES (AUNPS) IN THE RADIO-SENSITIZATION OF GLIOBLASTOMA CELLS

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BACKGROUND-AIM

Glioblastoma multiforme (GBM) is the most malignant form of primary brain tumour, with extremely poor prognosis due to bad response to therapeutic regimens. Ionizing radiation (IR) has been identified as a crucial treatment for GBM following surgical resection to improve overall survival. Unfortunately, radiotherapy resistance is a frequently observed phenomenon in affected patients. The mechanisms underlying the intrinsic radio-resistance in GBM are multifactorial, although altered DNA damage response seems to be the most crucial operator in the outcome to IR exposure.

METHODS

In the present work we are investigating the effectiveness of a novel approach to radio-sensitize GBM cells through the use of gold nanoparticles (AuNPs). AuNPs are promising radio-sensitizing agents due to their high biocompatibility and ability to be synthesized with various shapes and structures. AuNPs act by photothermal therapy (PTT), an efficient method of inducing localized hyperthermia aiming to selectively kill tumor cells.

RESULTS

In this work, AuNPs, specifically nanoprisms (NPrs), have been tested in two GBM cell lines: U87MG stabilized cell line and a primary cell line named GBM3. Preliminary data show that AuNPrs alone at low concentrations have no toxic effects in both GBM cell lines used, where AuNPrs demonstrated an efficient cytosolic internalization. More importantly, the combination of AuNPrs with increasing IR doses (2Gy-8Gy) showed a greater reduction in cellular viability and colony formation when compared with samples treated with IR alone.

CONCLUSIONS

This suggests that AuNPrs are able to weaken cells thus making them more susceptible to lower doses of IR. Combination therapy based on AuNPrs and subsequent low-dose IR could be considered a promising alternative to standard GBM treatment involving much higher IR doses (60Gy).

P057

GLUTAMINE ADDICTION OF MULTIPLE MYELOMA SHAPES A PRO-TUMOUR BONE MARROW NICHE

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BACKGROUND-AIM

In multiple myeloma (MM) malignant plasma cells proliferate in the bone marrow (BM), causing bone lesions characterized by a decreased number of osteoblasts (OB) and increased adipocytes. MM is the only human cancer to be both glutamine(Gln)-addicted and Gln-auxotroph (Bolzoni, Chiu et al, Blood 2016). This metabolic feature profoundly modifies patients' BM lowering Gln and increasing glutamate (Glu) levels. This peculiar environment impairs OB differentiation of mesenchymal stromal cells (MSCs) by limiting Gln-dependent asparagine (Asn) synthesis (Chiu et al, Cancers 2020). Although adipocytes metabolically sustain MM growth, the effects of MM metabolic anomalies on BM adipocyte differentiation are still unknown.

METHODS

Primary human BM MSCs (n=10) and human MM cell lines (HMCLs: RPMI8226, JJN3, MM1.S, U266) were grown in DMEM. MSCs were incubated in adipogenic medium (0.5mM IBMX, 5μM indomethacin, 50μM dexamethasone and 10mg/ml human insulin) for 14-days. Adipocytic differentiation was checked from gene expression and Oil Red O staining. Conditioned media of HMCLs was collected daily.

RESULTS

Stromal cells, but not malignant plasma cells, were strongly positive for Glutamine Synthetase (GS) in a series of BM biopsies of MM patients. During adipogenic differentiation, GS expression increased, while Glutaminase 1 decreased, suggesting a progressive independence of adipocytes from extracellular Gln. Lipogenesis and the expression of adipocytic markers PPARG, LEP, ADIPOQ, FASN, FABP4, CES1, ACLS1 was markedly increased in Gln-depleted MSCs compared with Gln-supplemented counterpart. This effect has been confirmed also under hypoxic conditions and was partially inhibited by Asn supplementation. Early adipocyte differentiation correlated with the induction of a nutritional stress response, which was further enhanced under Gln shortage, when intracellular Gln and Glu decreased, respectively, by 40% and 80%. Lastly, conditioned medium by all HMCL tested markedly enhanced adipocytic markers in MSCs, an effect hindered by Gln supplementation.

CONCLUSIONS

MM metabolism skews MSC differentiation from osteogenesis to adipogenesis, thus contributing to organize a pro-tumor BM niche. Asn supplementation counteracts this effect suggesting novel approaches to prevent MM bone pathological remodeling.

P058

QUERCETIN EXERTS A SENOLYTIC EFFECT ON DOXORUBICIN-INDUCED SENESCENT FIBROBLASTS BY REDUCING AUTOPHAGY, THEREBY PREVENTING THE PRO-TUMOR EFFECT ON OSTEOSARCOMA CELLS

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BACKGROUND-AIM

Cellular senescence is a tumour-suppressive mechanism contributing to aging and age-related diseases. Among the strategies developed to remove senescent cells (SCs), senolytic drugs are the most promising, although the mechanisms of action need to be defined. In this study, we used Quercetin as a senolytic agent on Doxorubicin (Doxo)-induced senescent fibroblasts, highlighting a new potential mechanism through which it killed SCs and reducing their pro-tumour effects on osteosarcoma cells (U2OS).

METHODS

(Doxo)-induced senescent fibroblasts (WI-38) were treated with Quercetin with or without mTOR inhibitor, PP242, or an inhibitor of endoplasmic reticulum (ER) stress, 4-PBA. To assess the reduction of SCs, we evaluated some senescence markers, intracellular calcium concentration with Fluo-4-AM dye, and cell death by Annexin/7AAD flow cytometry assay. Autophagy markers (Beclin-1, LC3-I/II, p62) were analyzed by WB and immunofluorescence. The presence of XBP1 spliced mRNA was evaluated by loading the products of RT-PCR on agarose gel. We investigated colony formation capacity and invasiveness of U2OS treated with fibroblasts conditioned media (CM).

RESULTS

Nowadays, it is widely known that the excess production of SASP factors in SCs can lead to ER stress, which is counteracted by increased autophagy. Our results showed that Quercetin kills selectively ~30% of senescent fibroblasts by reducing autophagy and exposing SCs to ER stress, which drives cell death. Indeed, senescent fibroblasts treated with Quercetin reduced autophagy markers and expressed XBP1 spliced mRNA, a marker of ER stress. This new mechanism of action of Quercetin was confirmed by separately combining the flavonol with PP242 and 4-PBA, which, by increasing autophagy and blocking ER stress, respectively, eliminated its senolytic effect. Furthermore, our results showed that the partial senolysis operated by Quercetin was sufficient to reduce the aggressive behaviours of U2OS cells induced by CM.

CONCLUSIONS

Our data prove the efficacy of Quercetin on Doxo-induced senescent fibroblasts, highlighting a new potential mechanism of action of the flavonol and demonstrating that even a partial reduction of SCs (30%) could lead to beneficial effects against age-related diseases, such as cancer.

P059

EFFECTS OF Aβ OLIGOMERS ON HUMAN ASTROCYTES FROM HEALTHY SUBJECTS AND ALZHEIMER'S DISEASE PATIENTS

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BACKGROUND-AIM

Alzheimer's disease (AD) is a neurodegenerative disorder whose incidence increases with advanced age and disproportionately affects women. The presence of beta-amyloid (Aβ) plaques seems to be one of the pathological features of AD. Soluble oligomers of $A\beta_{1-42}$ protein are the most neurotoxic, impacting neurons and other brain cells involved in CNS homeostasis. Among these, astrocytes could be a target and play a role in AD onset and exacerbation. We studied the effects of $A\beta_{1-42}$ oligomers on human primary astrocytes of AD (AD-astrocytes) patients and healthy subjects (H-astrocytes) to understand if astrocytes from affected and unaffected subjects could be differently sensible to the treatment. We investigated cell death and senescence induction by $A\beta_{1-42}$ oligomers, two processes involved in neuropathogenesis and neuroinflammation. We also studied gender differences, evaluating whether astrocytes from females react differently than astrocytes from males after treatment with $A\beta_{1-42}$ oligomers.

METHODS

Cells were treated with $A\beta_{1-42}$ oligomers at various time points. The ability of Aβ to interact with the cells was assessed by confocal microscopy and to evaluate intracellular Ca^{2+} levels using Fluo-4-AM dye in flow cytometry. Cellular viability and apoptosis were detected using the MTT assay and Annexin V/7AAD flow cytometry assay, while senescence was determined by analysing the expression of several senescence markers.

RESULTS

Findings showed that the internalisation of $A\beta_{1-42}$ oligomers and increased intracellular Ca^{2+} levels were similar in AD- and H-astrocytes. We observed that H-astrocytes underwent apoptosis, while a comparable number of AD-astrocytes entered senescence. Our results regarding gender differences indicated that among AD-astrocytes, those from females showed a significantly higher number of senescent cells than males (30.4% and 19.2%, respectively).

CONCLUSIONS

Our findings suggest that AD- and H-astrocytes take in $A\beta_{1-42}$ oligomers, causing a similar increase in intracellular Ca^{2+} levels. H-astrocytes undergo apoptosis, while AD-astrocytes enter a senescent state. The induced senescence in AD-astrocytes could contribute to neuroinflammation, leading to the progression and worsening of the disease, particularly in women.

P060

UNRAVELLING ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF OLEUROPEIN ON IMMUNOSENESCENCE AND INFLUENZA VACCINE RESPONSE IN OLDER ADULTS

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BACKGROUND-AIM

Oleuropein (OLE) is a polyphenol found in Olive leaves and fruits [1]. It has demonstrated anti-inflammatory, antioxidant, and pro-apoptotic properties, making it a potential nutraceutical compound for anti-aging strategies [1-4]. The research aimed to address the capacity of OLE to contrast the decline of the immune response in the elderly, characterised by immunosenescence and inflamm-aging.

METHODS

A cohort of 52 healthy subjects was recruited from the Improved vaccination Strategies for Older Adults (ISOLDA) between October and December 2020; blood was drawn before the administration of an anti-influenza vaccination (Flucelvax® Tetra) (T0), after 21-28 days (T1) and 55-60 days (T2) from the vaccination. Peripheral Blood Mononuclear Cells were obtained through density-gradient centrifugation, cultured with/without OLE, and with/without BIRB796 (a p38MAPK inhibitor). A combined condition of OLE and BIRB796 was also tested. The viral stimulus was provided by viral peptides, to recall the immune response to the virus in the T-cell populations in culture and to analyse T-cells-producing Tumor Necrosis Factor (TNF)-α, Interferon (IFN)-γ, and Interleukin (IL)-10 by flow cytometry.

RESULTS

The immunophenotype analysis showed an increase of T central and effector memory cells in both young and old individuals, although without statistical significance. Recalling the T-cell viral response in vitro, with influenza-specific viral peptides, old adults had higher frequencies of CD4+ IFN-γ+/TNF-α+ T cells at T1 than younger individuals. However, introducing OLE and BIRB796 reduced these differences, indicating that these compounds moderated the heightened T cell activation in old individuals. Specifically, BIRB796 decreased CD4+/CD8+TNFα+ T cells, alone or combined with OLE, suggesting they helped modulate the inflammatory response post-vaccination.

CONCLUSIONS

The study demonstrated that OLE, with/without BIRB796, has significant immunomodulatory effects, potentially enhancing the immune response and reducing inflammation in older adults following vaccination. These findings support the potential of OLE as a nutraceutical compound in improving the immune response and managing age-related immune decline.

P061

ONSET AND PROGRESSION OF IDIOPATHIC PULMONARY FIBROSIS: A ROLE FOR HLA MOLECULES

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BACKGROUND-AIM

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing interstitial lung disease of unknown cause with a very poor prognosis. IPF is characterised by alveolar damage and inflammation, deposition of extracellular matrix and distortion of lung architecture which ultimately results in respiratory failure. These modifications lead to restrictive lung disease, interfering with both gas exchange and lung compliance. However, the pathophysiology and the pathogenetic mechanisms underlying IPF remain poorly understood. Genetic variants in the Human Leukocyte Antigen (HLA) genes has been associated with inflammatory and respiratory diseases, including IPF. Taking advantage of the opportunity to study a population with low genetic variability such as the Sardinian one, our aim was to clarify the influence of HLA molecules on the onset and progression of the idiopathic pulmonary fibrosis.

METHODS

We compared the immune-genetic and phenotypic characteristics of 103 IPF patients with varying degrees of severity of the disease and 303 healthy controls from Sardinia (Italy). The genomic DNA was extracted from peripheral blood mononuclear cells following standard methods. All samples were genotyped at high resolution for the alleles at HLA-A, -B, -C, -DR and -G loci using Next-generation sequencing (NGS) AlloSeq Tx17 (CareDx) method based on Hybrid Capture Technology and performed on the Illumina platform. The data was analyzed using the AlloSeq Assign® software (v.1.0.2).

RESULTS

The analysis of HLA allele frequencies revealed an overlap between IPF patients and controls, with few significant differences. However, HLA allele frequencies differed in relation to the severity of IPF, resulting enriched the HLA-B*40:02:01 allele in patients with severe forms compared to controls and patients with mild disease.

CONCLUSIONS

The HLA-B*40:02:01 allele has been associated with several pathological conditions, suggesting a possible role in the susceptibility and progression of IPF. These genetic variations in HLA alleles could affect the control of the immune system response, leading to chronic inflammation in lung tissue, one of the main pathogenetic mechanisms of IPF.

P062

ROLE OF GLUTAMINE AND FATTY ACIDS IN THE METABOLIC REPROGRAMMING OF ANOIKIS-RESISTANT MELANOMA CELLS

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BACKGROUND-AIM

Among the several new features acquired by malignant cells during their progression, the circulating tumor cell (CTC) phenotype represents one of the most crucial to establishing a terminal metastatic disease, the principal cause of cancer-related mortalities. Indeed, the presence of CTC in carcinoma-bearing patients is considered a prognostic marker for cancer progression. Early during the formation and growth of a primary tumor cells are released from the primary site into the bloodstream and the resistance to detachment-induced apoptosis, known as anoikis, is a mechanism necessary for the survival and dissemination of CTC.
Here, we selected melanoma cells resistant to anoikis, and we studied their metabolic reprogramming, to identify new metabolic targets of CTCs.

METHODS

Subpopulations of melanoma cells expressing a high anoikis-resistant phenotype were selected by three consecutive rocking exposures in suspension and studied for their phenotypic and metabolic characteristics.

RESULTS

Anoikis-resistant cells displayed a higher ability to grow in suspension on agarose-covered dishes compared to control cells, and higher cell viability and colony formation capability after a further step in rocking condition. They showed an epithelial-to-mesenchymal transition associated with high invasiveness and a stemness-like phenotype. Anoikis-resistant melanoma cells in suspension showed a metabolic reprogramming from a characteristic glycolytic metabolism toward a more oxidative metabolism based on the use of glutamine and fatty acids, while re-adhesion on dishes reversed the metabolism to glycolysis. The treatment with metabolic inhibitors highlighted the effectiveness of rotenone (a mitochondrial electron transport chain complex I inhibitor), BPTES (a glutaminase inhibitor), SSO (a fatty acid transporter inhibitor), and etomoxir (a CPT1 inhibitor) in reducing the viability and the colony formation ability of cells capable of surviving in suspension, confirming the dependence of their metabolism on oxidative phosphorylation, using glutamine and fatty acids as the most important fuels.

CONCLUSIONS

This finding opens up new therapeutic strategies based on metabolic inhibitors of fatty acid oxidation for the treatment of CTCs and melanoma metastases.

P063

PROTECTIVE ROLE OF SHIGA TOXIN 1 IN THE EXPERIMENTAL PATHOGENESIS OF SHIGA TOXIN 2-RELATED HEMOLYTIC UREMIC SYNDROME

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BACKGROUND-AIM

Once released into the bloodstream, Shiga toxins (Stx) interact with platelets and leukocytes stimulating them to form aggregates and to release pathogenic extracellular vesicles (EV) containing Stx. These EV are considered the trigger producing the transition from bloody diarrhea to life-threatening hemolytic uremic syndrome (HUS) during human infections by Stx-producing Escherichia coli (STEC). In children, HUS is characterized by hemolytic anemia, thrombocytopenia and acute renal failure. The risk of a STEC-infected patient developing HUS varies significantly depending on the Stx type produced by the bacteria: 0% for Shiga toxin 1 (Stx1) or 24% for Shiga toxin 2 (Stx2). However, in patients infected by STEC producing both toxins the risk is halved.

METHODS

Human blood was challenged with nanomolar concentrations of Stx2, Stx1 or both toxins and the formation of leukocyte/platelet aggregates was evaluated by direct-flow cytometric analysis. Then, pathogenic blood cell-derived EV were isolated, their number and size determined by nanoparticle tracking analysis and their proteins characterized by capillary Western blotting.

RESULTS

Stx2 stimulated the production of a greater number of monocyte/platelet (CD14+/CD41+, $p<0.01$) or neutrophil/platelet (CD16/CD41, $p<0.01$) aggregates and of larger EV (8.5×10^{10}/ml) compared to Stx1 (3.3×10^{10}/ml) or controls (3.7×10^{10}/ml). Stx2-induced EV showed a higher amount of the vesicular marker Alix (214.2% ± 68.9, $p<0.05$) compared to control EV, whereas Stx1 induced a non-significant stimulation. Surprisingly, the concomitant presence of Stx1 and Stx2 reduced the formation of both monocyte/platelet and neutrophil/platelet aggregates ($p<0.001$) and the total number of EV (2.1×10^{10}/ml), in particular of the large (>200 nm) EV population. The expression of Alix (35.2 ± 30.5, $p<0.05$) was strongly reduced in Stx1+Stx2-induced EV compared to the sum of the values obtained in Stx1-induced EV and Stx2-induced EV. Notably, the amount of Stx2 significantly decreased in Stx1+Stx2-induced EV (25.4 ± 44.0, $p<0.05$) with respect to Stx2-induced EV.

CONCLUSIONS

These findings suggest that in STEC-infected children the presence of Stx1 mitigates the toxic action of Stx2, thus explaining the lower risk to develop HUS in patients infected by Stx1+Stx2-producing STEC.

P064

Aβ-INDUCED SENESCENCE IN ASTROCYTES FROM ALZHEIMER'S DISEASE PATIENTS CONTRIBUTES TO NEURONAL DEATH THROUGH SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE (SASP) AND METABOLIC CHANGES

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BACKGROUND-AIM

Aging is the main risk factor for Alzheimer's disease (AD). One theory suggests that accumulating soluble $A\beta_{1-42}$ protein oligomers (Aβ) contribute to AD onset. These Aβ are toxic for neurons and other brain cells, as astrocytes, that play crucial roles in CNS homeostasis. In chronic stress and aging, astrocytes can enter senescence, changing their metabolism and developing senescence-associated secretory phenotype (SASP), leading to neuron death. We studied SASP factors and metabolic enzymes in human primary astrocytes of AD patients (AD-astrocytes) and healthy subjects (H-astrocytes) of both sexes exposed to Aβ. Moreover, we evaluated the effects of astrocyte-conditioned medium (CM) on differentiated SH-SY5Y.

METHODS

AD-astrocytes and H-astrocytes were treated with Aβ, and after 120h, we assessed senescence primary markers, SASP factors and metabolic enzyme expression by WB, RT-PCR and Quantibody array. We tested the effect of treated-astrocytes CM on SH-SY5Y, evaluating at 24h viability and apoptosis by MTT and Annexin V/7AAD flow cytometry assay

RESULTS

Aβ oligomers treatment caused a senescent phenotype in AD-astrocytes, particularly in females, while H-astrocytes underwent apoptosis. The analysis of SASP factors showed variations in several molecules in both AD- and H-astrocytes. Additionally, we investigated astrocyte metabolism by analysing glucose and lactate metabolic enzymes critical for neuron survival. We observed that treated AD female astrocytes showed reduced expression of LDH A and MCT4 and increased levels of HK2 and G6PD compared to controls and treated AD male astrocytes. These preliminary data suggest that treated female astrocytes enhance glycolysis enzyme expression but reduce lactate production and export; conversely, they increase in AD male astrocytes. Moreover, data showed that after 24h, only the CM of AD-treated astrocytes induced apoptosis in SH-SY5Y cells

CONCLUSIONS

Preliminary data suggest that despite the SASP factor levels in AD-senescent astrocytes not being as remarkable as expected, CM from senescent astrocytes still causes SH-SY5Y cell death, emphasising their toxic role. The metabolic changes in AD-astrocytes after Aβ treatment suggest that factors beyond the studied SASP components may harm differentiated SH-SY5Y. Further investigations are required.

P065

MACROPHAGE-DERIVED IRON IN CHOLANGIOCARCINOMA: EFFECT ON TUMOR GROWTH AND MICROENVIRONMENTAL FEATURES

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BACKGROUND-AIM

Dysregulation of iron metabolism can contribute to both tumor initiation and progression. We recently showed that disrupted iron metabolism correlate with faster growth rate and shorter survival in patients with cholangiocarcinoma (CCA), a devastating cancer characterized by a very poor prognosis. Notably, the abundant stromal tissue of CCA includes tumor associated macrophages (TAM), that are relevant for iron redistribution to tumor and stromal cells in the tumor microenvironment.

METHODS

To investigate if TAM-iron released promotes CCA growth we genetically induced the CCA in mice with myeloid-cells specific loss of Fpn (KO) and their littermate controls (WT) using an approach relying on transposon-mediated stable integration of the AKT and Yap oncogenes delivered to hepatocytes by hydrodynamic tail vein injection. The effect of the lack of macrophages-derived iron on tumor and stromal cells was characterized at different time points (2, 3 and 5 weeks post hydrodynamic injection). Tumor histological analysis was performed by haematoxylin/eosin staining and the presence of tumor lesions was assessed by CK19 staining. Some CCA stromal features, including the presence of blood vessels and collagen deposits were evaluated by immunofluorescence, using antibodies against CD31 and collagen type I.

RESULTS

We observed an earlier onset of CCA in WT mice. In fact, 3 weeks post the injection, the focal and nodular formations of CK19+ tumor cells are smaller in KO than in WT mice. In both WT and KO mice we also observed the presence of enlarged lipid-rich hepatocytes, a general feature of AKT-overexpressing livers. At 5 weeks the liver was almost entirely affected by CCA in all mice, making the differences between the two types of mice less noticeable. Immunofluorescence staining also demonstrated the presence of different stromal features in WT and KO-derived CCA, with the former characterized by an enhanced presence of blood vessels and higher levels of collagen I, a marker of activated fibroblasts. These different stromal features are indicative of a more aggressive CCA progression in WT mice than in KO ones.

CONCLUSIONS

Our data suggest that macrophage-derived iron could play a key role in CCA development by facilitating the establishment of a tumor supporting microenvironment.

P066

HERG1/BETA1 INTEGRIN COMPLEX IS INVOLVED IN TUMOR PROGRESSION: BREAST, COLORECTAL AND PANCREATIC TUMOR-ON-CHIP MODELS IN A MICROFLUIDIC SYSTEM

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BACKGROUND-AIM

This study aims to develop 3D cell cultures of PANC-1, MDA-MB-231, and HCT-116 cell lines to more accurately replicate the in vivo tumor environment. The goal is to test the efficacy of specific blockers and recombinant antibodies in a controlled microfluidic system, enhancing the relevance and precision of the experimental results. A particular focus is placed on the hERG1/beta1 integrin complex, known for its role in tumor progression, and the potential to test specific blockers and antibodies targeting this complex.

METHODS

3D cell cultures of the aforementioned cell lines were established and maintained in a microfluidic system, which allowed for precise control of the cellular microenvironment. The system was used to administer specific blockers (E4031) and recombinant antibodies, particularly targeting the hERG1/beta 1 integrin complex. Cell viability and cytotoxicity were assessed using Calcein-AM/Propidium Iodide (PI) staining to distinguish live and dead cells and lactate dehydrogenase (LDH) assays to measure cell damage and death.

RESULTS

The results from both Calcein-AM/PI staining and LDH assays were consistent, indicating that while the blocker E4031 exhibited a noticeable effect on cell viability and cytotoxicity, the recombinant antibodies demonstrated a significantly more potent impact. Both assessment methods corroborated that the antibody had a stronger influence on reducing cell viability and increasing cytotoxicity compared to E4031, highlighting its potential as a more effective therapeutic agent against the studied cancer cell lines.

CONCLUSIONS

This study successfully developed 3D cell cultures of PANC-1, MDA-MB-231, and HCT-116 within a microfluidic system, providing a more physiologically relevant model for testing cancer therapies. The findings highlight the potential of using advanced cell culture techniques and microfluidic technologies to develop more effective cancer treatment strategies. Additionally, the targeted investigation of the hERG1/beta1 integrin complex underscores its critical role in tumor progression and the potential benefits of specific blockers and antibodies targeting this complex.

P067

EFFECTS OF N6-ISOPENTENYLADENOSINE (IPA) ON M1-POLARIZATION OF GLIOMA ASSOCIATED MICROGLIA IN GLIOBLASTOMA

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BACKGROUND-AIM

Glioblastoma (GBM) is one of the most aggressive types of brain cancer. Increasing evidence demonstrates that GBM cells establish a symbiotic interaction with the surrounding tumor microenvironment (TME), hijacking multiple cell types to fuel their growth. In this landscape, glioma-associated microglia, representing one-third of the tumor mass, are induced in a M2-immunesuppressive state, contributing to poor therapeutic outcome [1]. N6-isopentenyladenosine (iPA) is a natural molecule with well-known anti-glioma effects, carried out mainly through the downregulation of the mevalonate pathway. iPA has already been shown to have an immunomodulatory effect, due to its ability to selectively promote the activation of human-resting NK cells [2].

METHODS

We analyzed the expression of markers associated with the M1 and M2 state following treatment with iPA at a sub-lethal dose (1 μM) in order to collect preliminary data on the effects of this molecule on HMC3 cell line.

RESULTS

Here, we evaluate the effects of iPA on the crosstalk glioma-microglia, with particular attention on the secreted factors implicated in this molecular interaction. We observed that iPA (0,1 to 5 μM) had no effects on the viability of microglial cell line HMC3. We observed that HMC3 treated with sub-lethal doses of iPA (1 μM) showed a M1 pro-inflammatory phenotype, reflected by the increase of M1 markers (iNOS, CD11b, IL-6) and reduction of M2 markers (ARG1, TGFβ1, IL-10).

CONCLUSIONS

Altogether, these preliminary data suggest a new potential role for iPA, providing a rationale for future investigation on the molecular mechanisms underlying iPA action on microglia reprogramming.

P068

HARNESSING SILVER NANOPARTICLES TO TARGET BREAST CANCER: UNVEILING P38 MAPK-DRIVEN CYTOTOXIC MECHANISMS IN TRIPLE NEGATIVE AND HER2-POSITIVE BREAST CANCER CELL

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BACKGROUND-AIM

Breast cancer (BC) is a leading cause of death among women worldwide. Unfortunately, traditional therapies are still limited because of the onset of collateral effects in patients and of frequent tumor resistance to therapies. In this study we propose the use of silver nanoparticles (AgNPs) as a promising powerful tool to kill BC cells.

METHODS

AgNPs were synthesized and characterized both with spectroscopy UV-ris and FTRI, and NanoSight. Moreover, electron transmitted microscopy (TEM) was performed on AgNPs. Their effects on MDA-MB-231 and BT474 cell lines were analyzed with MTT assay, colony formation assay, invasion and migration assay and vascular mimicry. Molecular mechanisms caused by AgNPs were further investigated with Real Time PCR, Western Blot analysis and confocal microscopy.

RESULTS

Cell viability of MDA-MB-231 (triple negative – TNBC- metastatic cell line) treated with increasing doses of AgNPs for 24, 48 and 72h resulted significantly reduced and same results were obtained for the evaluation of long-term cell growth with colony assay. Alike, cell viability and long-term cell growth of BT474 (HER2 positive cell line) treated with AgNPs at the same time point resulted in a moderate cytostatic/cytotoxic effect. We demonstrated that AgNPs were able to cause the phosphorylation of P38 MAPK in both cell lines, through the production of ROS and, consequently, lipid peroxidation. Phosphorylation of P38 MAPK due to AgNPs administration was able to induce the activation of CHOP, ATF4 and GRP78, transcription factor related to ER stress and to induce the activation of cytochrome C and caspase 9, together with the increased expression of 21. Adezmapimod (SB 203580), a selective P38 MAPK inhibitor, was used to confirm that the observed responses were specifically mediated through the P38 MAPK pathway.

CONCLUSIONS

Based on our results, we conclude that AgNPs effectively induce ER stress and apoptosis via the intrinsic pathway. This study highlights the therapeutic potential of AgNPs for treating both TNBC metastatic and HER2-positive breast cancer cell lines. Our findings suggest that AgNPs could enhance existing therapies, offering a promising new approach in breast cancer treatment.

P069

PRIMARY LIVER CANCER-DERIVED ORGANOIDS FOR DRUG SCREENING AND DISEASE MODELLING

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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 achieve a better understanding of the molecular and cellular processes behind PLC development and also provide high-throughput experimental techniques to assess the treatments efficacy.
The aim of this study was to treat PLC patient-derived and steatosis-induced organoids with different substances, screening a library of molecules which experimentally interfere with the viability of tumour cells, in order to identify possible useful drugs against PLC.

METHODS

In order to develop organoids, tumor and non-tumor biopsies were minced and digested in small cell clusters that are then seeded into Matrigel. After characterization using immunofluorescence and qPCR techniques, PLC-derived and healthy organoids were treated with a different dosage of each selected compound for 72h, before measuring cell viability. We also studied possible underlying PLC processes, which are potentially targets of these substances.

RESULTS

We developed and characterized PLC-derived and healthy organoids, evaluating the morphological characteristics and revealing the presence of typical markers. Subsequently, as an initial screening, for each patient-derived organoids, we tested their sensitivity to 5 anti-cancer compounds, including drugs in clinical use or development. In particular, we started to analyze three small molecules targeting the Voltage Dependence Anion-selective Channel isoform 1 (VDAC1), showing a significant dose-dependent manner decrease of viability in tumor cells. In addition, we studied the effect of a natural-derived metabolite Usnic Acid, underlying a reduction of cell viability at high concentration. We also treated organoids with L-Asparaginase with no relevant effects. Moreover, we reveled a protective effect of 1-Piperidine Propionic Acid (1-PPA) in steatosis-induced organoid.

CONCLUSIONS

We developed and characterized a well-defined PLC in vitro model that allowed us to study the effect of multiple substances as potential novel therapy strategies.

P070

PRO-OXIDANT ACTIVITY OF FRUCTOSE IN CACO-2 INTESTINAL CELL MONOLAYERS

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BACKGROUND-AIM

Fructose is a simple monosaccharide sugar found in many foods and present above all in a large variety of fruits but also honey, balsamic vinegar and carbonated drinks. Recently, it has been suspected to be one of the main risk factors for the onset of inflammatory bowel diseases (IBDs) by altering cellular redox state, promoting the production of pro-inflammatory mediators and leading to an alteration of intestinal permeability. In this regard, the aim of this study was to investigate the effects of fructose on the intestinal epithelial barrier integrity in relation to its pro-oxidant activity.

METHODS

Experiments were conducted in vitro using monolayers of differentiated Caco-2 intestinal epithelial cells. In this model we tested fructose at different concentrations (1 – 50 mM) to evaluate the alteration of cell monolayers permeability, through the measurement of transepithelial electrical resistance (TEER), its cytotoxicity (MTT assay), the intracellular production of radical oxygen species (ROS) (DFCH-DA test) and the amount of intracellular reduced (GSH) and oxidized glutathione (GSSG) through HPLC-ECD. We also analysed the modulation of the redox sensitive MAP kinase ERK1/2 via Western blot and the release of nitric oxide (NO).

RESULTS

Fructose (25-50 mM) induced a significant increase of the monolayer permeability after 2 h of incubation. It did not induce cell death but showed pro-oxidant activity from the lowest concentration tested (1 mM) and it was effective in inducing ERK1/2 phosphorylation. The GSH/GSSG ratio was decreased at early stages (2 h) and increased after long incubation (24 h) due to cellular response to oxidative stress. An increase in NO production in presence of fructose was also observed.

CONCLUSIONS

Herein we highlighted the deleterious effect of fructose on the permeability of the intestinal epithelial barrier through its pro-oxidant property. These results encourage more in-depth investigation on the redox aspects of fructose toxicity and its contribution to the risk of developing IBDs.

P071

LOSS OF DDB2-PCNA PROTEIN INTERACTION IN IRRADIATED CELL DETERMINES THE ACQUISITION OF TUMOR-LIKE PHENOTYPE

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BACKGROUND-AIM

The DDB2 protein plays a crucial role in the initial step of global genome Nucleotide Excision Repair. Our previous research demonstrated that the interaction between DDB2 and PCNA is important for the efficient removal of DNA lesions. In fact, cells expressing DDB2 unable to bind PCNA ($DDB2^{PCNA-}$) show a delay in the NER process and exhibit tumor-like characteristics. Among these, the ability to form colonies after UVC exposure was observed.

METHODS

We performed clonogenic assay on soft agar on non-irradiated and UV-C irradiated (10 J/m^2) HEK293 cells, including both untransfected cells and those expressing either wild-type or mutated DDB2 protein. Two resistant clones (clone 1 and clone 2) were isolated from cells expressing the mutated protein and expanded. Flow cytometry, microscopy, and Western blot techniques were employed to evaluate protein expression levels; iCELLigence biosensor technology to assess adhesion capabilities, and Boyden chamber and wound healing assays to evaluate migratory behavior.

RESULTS

We investigated the clonogenic efficiency of the cells after treatment with different drugs. The clone 2 produced a higher number of colonies after cisplatin treatment and was more sensitive to HU. Additionally, both clones exhibited elevated levels of CD117, CD44 and OCT4, which are involved in different pathways.
Interestingly, the UVC-resistant clones showed reduced adhesion capabilities, faster gap closure in wound healing assay, but in the Boyden chamber assay, only clone 2 exhibited high migratory capability. Furthermore, Western blot analysis revealed increased expression of SNAI1, NF-kB and E-Cadherin, along with decreased expression levels of ZEB1 and Vimentin. Preliminary studies also indicated that the supernatant from cells producing the $DDB2^{PCNA-}$ protein could promote differentiation of THP-1 monocytes into macrophage.
Finally, all cell lines were able to form spheroids, thought at different time. To evaluate the three-dimensional structure of the spheroids, we analyzed proteins involved in their structural maintenance.

CONCLUSIONS

These data not only confirm a tumor-like phenotype in the $DDB2^{PCNA-}$ cell line but also highlight differences between the two clones. This heterogeneity warrants further investigation in future studies.

P072

MESENCHYMAL STROMAL CELLS TRADE OFF GLUTAMATE FOR GLUTAMINE TO SUSTAIN MULTIPLE MYELOMA CELLS GROWTH

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BACKGROUND-AIM

Besides sustaining neoplastic cell growth, deranged cancer metabolism also impacts other cell populations of the tumor microenvironment. In Multiple Myeloma (MM), malignant plasma cells are both glutamine (Gln)-addicted and Gln-auxotroph, leading to a partial Gln depletion and a glutamate (Glu) increase in patients' bone marrow (BM). These metabolic features force Glutamine Synthetase (GS) expression in Mesenchymal Stromal Cells (MSC) and impair their differentiation into osteoblasts (OB), thus favoring bone destruction.

METHODS

Primary human BM MSC from healthy donors and human MM cell lines were grown in RPMI1640 medium supplemented with 4mM Gln and 10% FBS. For OB differentiation, MSC were incubated in osteogenic medium for 14 days. Gln secretion and ^{13}C metabolite tracing was measured by mass spectrometry. ^{3}H-Glu influx was used to determine EAAT3 transporter activity. Cell viability was evaluated by the resazurin assay.

RESULTS

More than 50% of Glu in MM cells directly derives from Gln deamidation. Neoplastic plasma cells secrete substantial amounts of Gln-derived Glu in the extracellular space through the SLC7A11 transporter. MSC, but not OB, display sodium-dependent Glu uptake and a high expression of the EAAT3 Glu transporter. Consistently, public transcriptional profiles of BM biopsies of healthy donors or MM patients reveal that the expression of EAAT3 is higher in MSC compared to OB. In Gln-free conditions, MSC secrete higher amounts of Gln than OB, a phenomenon boosted by extracellular Glu supplementation The dependence of Gln secretion by Glu uptake and Gln synthesis is demonstrated by its suppression by either GS or EAAT3 inhibitors. In co-cultures, MSC increase the expression of the efflux Gln transporter SNAT5 and support MM cell viability under Gln shortage, an effect impaired by either the inhibition or the silencing of GS or EAAT3 in MSC.

CONCLUSIONS

Thus, in the MM BM niche, malignant plasma cells secrete Glu that is taken up by MSC that synthetize and secrete Gln, thanks to the activity of EAAT3 and GS, thus sustaining MM Gln auxotrophy. Several steps of these deranged pathways are sensitive to pharmacological inhibition and may constitute novel therapeutic targets to counteract MM growth in the BM niche.

P073

EXTRACELLULAR VESICLES CHARACTERIZATION IN PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY

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BACKGROUND-AIM

Hypertrophic cardiomyopathy (HCM) is a genetic disorder, diagnosed according to the presence of phenotypical traits of the myocardium. A specific biomarker which can associate with the severity of HCM is lacking. Extracellular Vesicles (EVs), nanoparticles released by cells into biological fluids, hold promise as accessible diagnostic tools, as their abundance and molecular composition can reflect the severity of cardiac diseases. We aim at characterising plasma-derived EVs isolated from 34 HCM patients and 21 healthy volunteers (CTR).

METHODS

The whole cohort underwent transthoracic echocardiography, whereas magnetic resonance and exome sequencing was performed on HCM patients. EVs were isolated via ultracentrifugation from plasma of both groups. Quantitative and qualitative assessments of EVs were performed by nanoparticle tracking analysis, transmission electron microscopy, Western blot, targeted and untargeted proteomic and FACS analyses. Plasma-derived EV subpopulations were characterised according to their cellular origin. Data are expressed as median and interquartile range (25^{th}, 75^{th}). Plasma levels of Galectin-3 were assessed via ELISA.

RESULTS

Most patients were male (65% HCM and 70% CTR) with a median age of 58 (49-70) years (HCM) and 48 (44-54) (CTR). Among HCM patients, mutations in the MYH7 and MYBPC3 genes were the most identified. The median maximum wall thickness (WTMax) in HCM was 16 mm (15-18) vs 9 mm (8-10) in CTR. Galectin – 3, an index of fibrosis, was significantly increased by 34 % in HCM patients compared to the CTRL group. Median EV concentration was reduced in HCM vs CTRL, respectively 3.3*109 EV/ml/cell count (2.4*109-5*109) and $4.6*10^9$ EV/ml/cell count (3*109-6*109). Among the 15 EV subpopulations analysed, those released from platelets (CD41a) were doubled in HCM patients vs CTR (median values: 36.5 EVs/μL vs 17.6 EVs/μL). A similar trend was found for EVs released from activated platelets (CD62P and CD40L). Negative associations were found between E/e', parameter of diastolic function, and cardiomyocyte-derived EVs (r= -0.232). The proteomic analysis of EVs shows an enrichment in proteins related to platelets adhesion and inflammation in HCM patients.

CONCLUSIONS

HCM patients present a peculiar phenotypic pattern of EVs that may associate with macroscopic and microscopic features of HCM.

P074

NINTEDANIB-CONTAINING DUAL CONJUGATE TARGETING αVβ6 INTEGRIN AS ANTIFIBROTIC AGENT IN A PRECLINICAL MODEL OF IDIOPATHIC PULMONARY FIBROSIS

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BACKGROUND-AIM

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease with unclear pathogenesis, potentially involving alveolar epithelial microlesions leading to abnormal re-epithelialization. Mediators like TGFβ, bFGF, and PDGF sustain fibrosis, while αVβ6 integrin receptor is a key factor in TGFβ activation through the recognition of RGD peptide. Despite treatment with antifibrotic drugs like the receptor tyrosine kinase (RTK) inhibitor Nintedanib, the disease continues to progress, and there is an urgent need of more effective treatments.
Here we evaluate the biological activity of a novel conjugate of Nintedanib with a specific ligand for αVβ6 integrin to selectively target fibrotic cells in order to dampening TGFβ release, and disrupting fibrosis progression.

METHODS

αVβ6 expression and conjugate internalization were evaluated in healthy and diseased fibroblasts using cytofluorimetric assay. Western blot assay was used to evaluate the expression of fibrotic markers in healthy fibroblasts primed with TGFβ and exposed to αVβ6-nintedanib conjugate. The inhibition of in vivo fibrosis was determined in a mouse model (bleomycin-induced), through histology and the analysis of Bronchoalveolar Lavage Fluid biomarkers (BALF).

RESULTS

We found that αVβ6 integrin is expressed in both healthy and diseased fibroblasts, with elevated levels in diseased cells. The conjugates were effectively internalized, and the treatment modulated fibrotic markers expression, highlighting αVβ6 as a potential target for fibrosis modulation. Interestingly, the conjugates significantly decreased in vivo fibrosis, as demonstrated by a reduction in fibrotic regions detected using micro-CT.

CONCLUSIONS

Combining a RTKI with an RGD peptide shows potential for enhancing IPF treatment by targeting fibrotic cells, TGFβ activation, and growth factor signaling. Future research should assess their impact on endothelial cells, macrophages, and long-term safety and efficacy in IPF patients.

P075

UNVEILING THE HAT4/NAA60 BIFUNCTIONAL GOLGI ACETYLTRANSFERASE EPIGENETIC ROLE IN LEUKAEMIA

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BACKGROUND-AIM

Protein acetylation is one of the most studied post-translational modifications, deeply involved in several biological processes. By modulating chromatin structural organization, gene expression and proteostasis, protein acetylation plays a pivotal role in epigenetic regulation in physiology and pathology. HAT4, also named NAA60/NatF/NAT15, is one of the newest N-term acetyltransferases endowed with biochemical features shared by both B-HAT and NAT enzymes. It is reported to be a bifunctional acetyltransferase modifying histone as well as non-histone proteins. It transfers acyl groups to both newly synthesized histone lysine and to substrates starting with Met-Lys/Met-Ala/Met-Val/Met-Met peptides. Moreover, HAT4/NAA60 shows a peculiar cell sublocalization. Intriguingly, it is localized in the Golgi membrane stacks facing the cytosol.
Due to its enzymatic features and unusual cell localization for an acetyltransferase member, a wide characterisation might represent a new challenge for cancer therapy, potentially looking at HAT4/NAA60 as a novel targetable epigenetic enzyme in leukaemia and extending the knowledge in solid tumours.

METHODS

Western blot, immunofluorescence, cell lines engeneering and FACS analysis have been performed.

RESULTS

Molecular investigations have been carried out in different leukaemia cell lines showing a variable expression at protein level and the presence of not yet characterised isoforms and conformers. Protein over-everpression and gene silencing stategies have been also followed for comparative phenotype studies. A peculiar protein distribution in the cell compartments has been observed. Particularly, an enlargment of the Golgi surface has been observed in the perinuclear and ER continous region (cis- Golgi) and in the citosol side (trans- Golgi) facing the cell membrane. The Golgi architecture seems to be stricIty related to HAT4/NAA60 protein expression thus reflecting its function as Golgi transmembrane protein (important for Golgi integrity) and bifunctional acetyltranferase as HAT and NAT enzyme.

CONCLUSIONS

The knowledge about HAT4/NAA60 biology represents an attractive topic in the biochemistry of the acetiltransferase family and in the field of personalized medicine. Our pioneristic studies on leukaemia cell models may pave the way to the elucidation of the molecular basis leading on the progression of peculiar biological events involving Golgi architecture and function in tumoural envirorments.

P076

EXPLORING THE EFFECTS OF CULTURE MEDIUM DERIVED FROM HEK293 CELLS EXPRESSING DDB2 PCNA- PROTEIN ON MONOCYTES DIFFERENTIATION

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BACKGROUND-AIM

DNA damage binding protein 2 (DDB2) is essential in the early step of DNA damage recognition in nucleotide excision repair (NER), a process activated by UV-induced DNA damage. DDB2 interacts with PCNA through a conserved sequence known as the PIP-box. This interaction is crucial for the proteosome-dependent degradation of DDB2 following the DNA damage recognition step. A mutation in the DDB2 PIP-box, disrupting this bond, results in defective DNA repair and in the induction of an aggressive cellular phenotype, which is also linked to the NF-kB induction.
The aim of this study is to investigate the possible influence of DDB2 on the inflammatory microenvironment and its role in monocyte differentiation.

METHODS

For this purpose, we used THP-1 monocytes, grown in culture medium derived from HEK293 control cells, HEK293 cells stably expressing $DDB2^{wild\text{-}type}$ protein, or DDB2 unable to interact with PCNA ($DDB2^{PCNA\text{-}}$). These cells were either non-irradiated or collected at various times points after UV-C irradiation ($10J/m^2$) to evaluate whether the conditioned medium contained molecules able to induce monocyte differentiation into macrophages.

RESULTS

First, we assessed cell morphology and adhesion at different incubation times using Gentian violet staining. THP-1 cells grown in medium derived from irradiated HEK 293 $DDB2^{PCNA\text{-}}$ cells, adhered and acquired an irregular shape, characterized by the presence of multiple extensions/filaments.
Second, we performed immunofluorescence analysis to examine the expression of CD80 and CD68, which are markers for monocytes and macrophages, respectively. The results showed that in THP-1 cells treated with the medium from both non-irradiated $DDB2^{PCNA\text{-}}$ cells and those collected 3 and 7 days post-irradiation, both the signal for CD80 and CD68 increased at the plasma membrane, the last one also at the intracellular compartment. In both cases, the cells progressively adopted a more irregular morphology.

CONCLUSIONS

We hypothesize that the differentiation of THP-1 cells in macrophages could be induced by specific molecules present in the medium, whose levels increase particularly in UV-irradiated $DDB2^{PCNA\text{-}}$ cells, suggesting a potential link between defect in DNA repair and inflammatory microenvironment.

P077

TARGETING SIRTUINS AS WEAPON TO SHUTDOWN NLRP3-INFLAMMASOME IN LEUKEMIA

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BACKGROUND-AIM

The NLRP3 inflammasome is a multiprotein complex that plays a critical role in the immune response by promoting the maturation and the secretion of inflammatory cytokines, such as IL-1β and IL-18. However, the dysregulation of the NLRP3 inflammasome has been linked to a variety of pathological conditions, including autoimmune diseases, metabolic disorders, and notably cancer. In the context of cancer, particularly leukemia, inhibiting the NLRP3 inflammasome is essential to counteract chronic inflammation. Therefore, targeting the NLRP3 inflammasome represents a promising therapeutic strategy to inhibit tumor growth and overcome resistance to current therapies. Recent research has focused on developing small molecules and biological agents that can specifically inhibit the NLRP3 inflammasome. These inhibitors aim to disrupt the assembly and activation of the inflammasome complex, thereby reducing the production of pro-inflammatory cytokines and mitigating the associated pathological inflammation.

METHODS

WESTERN BLOT (WB)
IMMUNOPRECIPITATION (IP)
IMMUNOFLUORESCENCE (IF)
FLOW CYTOMETRY (FACS)

RESULTS

In this study, we investigated the interaction between the core components of the NLRP3 inflammasome, specifically NLRP3 and ASC, under basal conditions in leukemic cells. Furthermore we evaluated the effects of the pan-SIRT-inhibitor MC2494 on the main components of NLRP3-inflammasome. Our results demonstrate that MC2494 effectively disrupts the NLRP3-ASC interaction, leading to a significant downregulation of inflammasome activity. These findings provide valuable insights into the potential of MC2494 as a therapeutic agent targeting the NLRP3 inflammasome and chronic inflammation in leukemia.

CONCLUSIONS

Our study demostrated that the pan-SIRT inhibitor MC2494 effectively disrupts the interaction between NLRP3 and ASC, leading to a substantial downregulation of NLRP3 inflammasome components. This disruption significantly reduces the production of IL-1β, which contribute to chronic inflammation in leukemia microenvironment. These results highlight the therapeutic potential of MC2494 as a novel anti-inflammatory and anti-cancer agent through NLRP3-inflammasome inhibition and sirtuin targeting.

P078

A NEW VASCULARIZATION STRATEGY FOR REGENERATIVE NANOMEDICINE: DUAL STEM CELL-BASED THERAPY STRENGHTENED BY PROANGIOGENIC GOLD NANOPARTICLE DELIVERY

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BACKGROUND-AIM

Cardiovascular disease (CVD) is a group of heart and circulatory diseases considered the first cause of death worldwide. New approaches promoting angiogenesis are urgently needed to increase therapeutic effects and reduce the risk burden associated with surgery for patients.
Both Mesenchymal stem cells (MSCs) and Endothelial colony-forming cells (ECFCs) hold significant promise as candidates for regenerative cell therapy of vascular injury: ECFCs because of their high clonogenic potential and ability to originate de novo blood vessels in vivo, MSCs due to their ability to differentiate into cardiovascular cells, antifibrotic activity and ability to undergo neo-vasculogenesis.
Despite nanomedicine is considered a novel and effective approach for targeted vascular disease treatment, its application in CVD is still challenging. The difficulty lies in the delivery of nanoparticles to specific blood vessel lesions. Since cotransplanting ECFCs into the grafts provides sufficient trophic support to MSCs, we hypothesized that MSC and ECFC coculture, used also as cargo of proangiogenic gold nanoparticles (AuNPs) tested in our laboratory, could potentiate the regenerative therapeutic effect of each one.

METHODS

To test the effect of AuNPs on ECFC ability to form vessel structures, in coculture with MSC, we performed capillary morphogenesis on Matrigel. 3D invasion assay with Boyden chamber and scratch assay were used to test invasion and migration capacity of the AuNP-ECFCs + MSC coculture, instead immunofluorescence assay to evidence vessel network formation trough CD31 and fibronectin staining. Then, to reflect the in vivo neo-vascularization process, 3D spheroid angiogenesis assay composed of AuNP-ECFCs + MSCs were developed.

RESULTS

AuNP-loaded ECFCs stimulated neo-angiogenesis and this effect is boosted by the presence of MSCs. Moreover, migration, invasion and enhanced angiogenic properties of AuNPs-enriched ECFCs in coculture with MSCs were also confirmed.

CONCLUSIONS

We propose a promising strategy to deliver therapeutic gold nanoparticles with pro-angiogenic features through their internalization in ECFCs that have an innate injured tissue-tropism. Therefore, to potentiate the regenerative therapeutic potential, we suggest a dual cell therapy AuNP-ECFCs + MSCs.

P079

MACROPHAGE POLARIZATION AND PROGNOSTIC IMPLICATIONS IN GLIOBLASTOMA

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BACKGROUND-AIM

Glioblastoma (GBM) is the most common and aggressive glioma in adults supported by a highly immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages (TAMs), which constitute about one-third of the TME cells, could affect survival and therapeutic response. This study aims to deepen the TAMs prognostic role by analyzing macrophages' polarization in a well-characterized series of GBM patients.

METHODS

Surgical specimens of 59 GBM adult patients were retrospectively selected, and where available relapsed tissue was also collected. Adjuvant radio- and chemotherapy, relapse, and cancer-related death times were annotated. Immunohistochemical expression of CD68, CD80, and CD163 was digitally assessed by HALO® v3.6 imaging analysis software. All statistical analyses were performed with SPSS 27.0 and GraphPad Prism v10.1.

RESULTS

A total of 23 (39%) patients relapsed and 54 (92%) died during the follow-up. Biomarker analysis showed higher levels of CD68 ($p<0.00019$), CD80 ($p=0.0421$) and CD163 ($p<0.0001$) in GMB tissues compared to peritumoral tissues, with significant CD163 upregulation ($p<0.0024$) in recurrent tumors tissue. CD163 were significantly higher in peritumoral ($p=0.0078$) and GMB tissue ($p<0.0001$) in relapses vs primary GBM. Spearman analysis supported positive correlation among these markers in primary and recurrent tissues. Prognostically, $CD163^{High}/CD80^{High}$ subgroup showed a lower overall survival (OS), (HR=2.24; 95% CI: 1.2-4.4; $p=0.027$) and progression-free survival (PFS), (HR=1.41; 95%CI: 0.8-2.5; $p=0.213$) compared to other subgroups.

CONCLUSIONS

This study confirms the intricacy and multifaceted GBM's microenvironment. It challenges the notion of macrophage polarization as a simple M1 or M2 dichotomy, despite the notable CD163 expression in GBM TME. Instead, it suggests a synergy between macrophage populations, by identifying a subgroup with high concurrent CD80 and CD163 expression as a negative prognostic factor for OS and PFS. These findings highlight TAMs as potential therapeutic targets and may lead to personalized oncology therapies.

P080

GLUTATHIONE TRANSFERASE OMEGA 1-1 (GSTO1-1) PROMOTES THE RESISTANCE OF CANCER CELLS TO TOPOTECAN BY MODULATING THE APOPTOSIS/RESISTANCE BALANCE.

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BACKGROUND-AIM

GSTO1-1 is an atypical member of the glutathione transferase superfamily with both reductase and thioltransferase activity, rather than the classic detoxification function. GSTO1-1 is highly expressed in a number of neoplasms and its levels increase along with cancer progression [1]. Studies performed in our laboratory have demonstrated that GSTO1-1 modulates cellular response against cisplatin [2] and As2O3 (ATO) [manuscript in preparation], suggesting the role of GSTO1-1 in general, rather than drug-specific, cellular response mechanisms. Topotecan is an anti-tumor drug with topoisomerase I-inhibitory activity used for the treatment of ovarian cancer, small cell lung cancer and cervical cancer. We have investigated such possible role(s) of GSTO1-1 in response to Topotecan treatments in in vitro experiments on cervical carcinoma derived HeLa cells.

METHODS

HeLa GSTO1-1 stable transfected (HeLaGSTO1+), HeLa control (HeLaCont) and HeLa CRISPR/Cas 9 ko (HeLaGSTO1-) cells were used to test the toxicity (Alamar) of increasing doses of Topotecan. Apoptosis (Hoechst), autophagy (LC3, IB), MAPKs activation (immunoblot) and GSTO1-1 localization (IHF) were evaluated.

RESULTS

The survival of HeLaGSTO1+ cells was higher than that of HeLaCont and HeLaGSTO1- cells at all concentrations tested, suggesting that GSTO1-1 expression can efficiently protect cells against Topotecan toxicity. Indeed, a transient nuclear translocation of GSTO1-1, the activation of survival pathways (Akt and ERK1/2) and the inhibition of apoptotic pathways (JNK1) were detectable in more resistant HeLaGSTO1+ cells.

CONCLUSIONS

Our results suggest that GSTO1-1 over-expression may contribute to protect cancer cells against Topotecan. The involvement of the same pathways associated with cell survival against cisplatin and ATO confirms a more general role of GSTO in defense mechanisms, i.e. not limited to drug-specific phase I or II detoxification reactions.

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2. Piaggi S, et al. Carcinogenesis. 2010; 31: 804

P081

MICRORNA AS A PROMISING MOLECULAR BIOMARKER FOR LIQUID BIOPSY IN BREAST CANCER

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BACKGROUND-AIM

Breast cancer (BC) is the most frequently diagnosed cancer among women. Mortality associated with BC is generally attributable to metastatic relapse, which swiftly leads to multi-organ failure. Recent advancements in translational medicine have concentrated on identifying novel biomarkers that can provide valuable insights into patient outcomes. A comprehensive analysis of circulating miRNAs can significantly enhance our understanding of tumorigenesis and facilitate the development of miRNA-based approaches for the prognosis, diagnosis, and treatment of breast cancer.

METHODS

21 plasma samples from BC patients were analyzed. Total RNA was extracted in automation and library preparation was carried out with the QIAseq miRNA Library kit (Qiagen). The molecular characterization of circulating miRNAs was performed by massive sequencing on an Illumina miSeq platform. Differential miRNA expression was conducted by the RNA-seq Analysis Portal (RAP) considering as significance a False Discovery Rate (FDR) value <0.1. NGS data were confirmed by RT-qPCR in 30 patients.

RESULTS

The study revealed the aberrant expression of 10 miRNAs between early breast cancer vs metastatic patients. In particular, miR-146a-5p, miR-126-5p, miR-122-5p, miR-16-5p, miR-142-3p, miR-223-3p, miR-103a-3p, miR-221-3p, miR-21-5p, miR-30d-5p were significantly (FDR<0.07) associated with an advanced disease. Likewise, higher levels of miR223-3p, miR146a-5p and miR148b-3p were observed in ductal vs lobular (FDR<0.1) tumor histotypes.
The deregulation of one key miRNA correlated with patients' metastatic pattern. The up regulation of miR126-3p was associated with the development of visceral metastases (FDR<0.05).
The expression profiles found are useful in stratify patients in more homogenous groups.

CONCLUSIONS

The results of the present study suggest that miRNA profiling could be used as new potential prognostic and predicting tool in breast cancer, overcoming the limitations of the validated biomarkers.

P082

TRKAIII PROMOTES PD-L1 EXPRESSION IN SH-SY5Y NEUROBLASTOMA CELLS.

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BACKGROUND-AIM

Neuroblastomas (NBs) are heterogeneous, aggressive, therapy-resistant embryonal tumours of neural crest origin. Alternative TrkAIII splicing of the TrkA neurotrophin receptor in NBs correlates with post-therapeutic relapse and advanced stage metastatic disease, is characterized by exons 6 and 7 skipping and expression of a variant receptor devoid of the extracellular D4 domain that accumulates in pre-Golgi membranes and exhibits ligand-independent activation. In NB cell models, intracellular TrkAIII activation induces pro-survival PI3K-AKT signaling, enhances stress and drug-resistance and augments primary and metastatic tumorigenicity. Immune evasion is an additional hallmark of tumor progression and PD-L1/PD1 immune checkpoint activation has been identified as a potential target in NBs. Considering this, and the correlation between alternative TrkAIII splicing and NB progression, we evaluated whether TrkAIII may also regulate PD-L1 expression, in an SH-SY5Y NB cell model.

METHODS

PD-L1 mRNA and protein expression were evaluated by RT-qPCR, RT-PCR, Western blotting and indirect immunofluorescence in stable pcDNA, TrkA and TrkAIII SH-SY5Y transfectants, under untreated conditions (all cell lines), following treatment with NGF (TrkA SH-SY5Y), treatment with DTT (TrkAIII SH-SY5Y) and in all cell lines following treatments with Trk, PI3K and MEK inhibitors. PD-L1 function was assessed by IL-2 ELISA in Jurkat/TrkAIII SH-SY5Y cell co-cultures.

RESULTS

Control and TrkA-SH-SY5Y transfectants exhibit similar PD-L1 expression. NGF enhances PD-L1 expression in TrkA-SH-SY5Y transfectants, which is prevented by Trk and PI3K but not MAPK inhibitors. TrkAIII SH-SY5Y transfectants exhibit elevated PD-L1 expression, which is also reduced by Trk and PI3K but not MAPK inhibitors and by TrkAIII siRNA. TrkAIII SH-SY5Y PD-L1 inhibits Jurkat cell IL-2 production in co-cultures.

CONCLUSIONS

The data confirm that NGF-activated TrkA and TrkAIII promote PD-L1 expression in SH-SY5Y NB cells via PI3K but not MAPK and confirm PD-L1 function in preventing T cells IL2 production. We propose, therefore, that promotion of PD-L1 expression in NB cell can be added to the oncogenic repertoire of TrkAIII and targeted by Trk and PI3K inhibitors.

P083

A TAQMAN-BASED QRT-PCR ASSAY FOR DETECTING TRKAIII EXPRESSION IN TUMOR TISSUE RNAS

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BACKGROUND-AIM

The oncogenic alternative TrkAIII splice variant, discovered in human neuroblastomas (NBs), correlates with post therapeutic relapse and advanced stage metastatic disease and is characterized by TrkA exons 6, 7 and 9 skipping. TrkAIII expression also correlates with advanced stage metastatic cutaneous malignant melanomas and Merkel cell carcinomas and is expressed by Pituitary neuroendocrine tumors. In NB models, TrkAIII accumulates in pre-Golgi membranes, mitochondria and centrosomes, within which it exhibits ligand-independent activation. In NB models, intracellular TrkAIII activation induces pro-survival PI3K/Akt signaling, an angiogenic MMP-9/VEGF/Tsp1 expression equilibrium, centrosome amplification and stress-induced metabolic adaptation, which enhance primary and metastatic tumorigenicity. In NB models, TrkAIII exhibits similar oncogenic activity to the TrkA fusion oncogene TrkT3, characterizing TrkAIII as an oncogenic alternative to TrkA gene fusion. This suggests that approved Trk inhibitors that elicit profound long-lived responses in cancers driven by Trk-fusion oncogenes, may also be efficacious in cancers that express TrkAIII, making their initial identification by way of an effective TrkAIII qRT-PRC assay, an important goal.

METHODS

Real-Time TaqMan-based TrkAIII qRT-PCR assays were developed, optimized and validated to evaluate TrkAIII expression in simple and complex RNA and cDNA mixtures (TrkA and TrkAIII cDNAs, stable TrkA and TrkAIII SH-SY5Y transfectant mRNAs and fresh and FFPE tumor tissue RNAs).

RESULTS

One of several TaqMan-based TrkAIII qRT-PCR assays, optimized for annealing/extension temperatures, DMSO and BSA concentrations, exhibited optimal amplification efficiency, dynamic range, reproducibility and sensitivity down to 102 copies of TrkAIII mRNA. This assay was selected for further development.

CONCLUSIONS

The TaqMan-based real time TrkAIII qRT-PCR assay selected in this study exhibits sufficient efficiency, reproducibility and sensitivity for routine evaluation of TrkAIII expression in complex RNAs from fresh and FFPE tumor tissues and, therefore, represents a novel preliminary method to identify advanced stage refractory tumors that exhibit TrkAIII expression, which may respond to approved Trk inhibitory therapy.

P084

ANTIBIOTIC AEROSOLIZATION PERTURBS THE TUMOR-ASSOCIATED MICROBIOTA IN THE LUNG AND INCREASES IMMUNOTHERAPY EFFICACY BY TRIGGERING T MEMORY STEM CELLS

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BACKGROUND-AIM

Gut microbiota controls the response to immune checkpoint inhibitors (ICIs) and intestinal dysbiosis impairs the immunotherapy effectiveness. Lungs harbor a resident microbiota which plays a role in regulating pulmonary immune tolerance. Tissue microbiota is modified by the presence of a growing tumor, but the effect of tumor-associated microbiota on ICIs blockade efficacy remains largely unexplored. Here, we evaluate the effect of the tumor-associated microbiota perturbation by antibiotics aerosol in a murine lung cancer model and its efficacy in improving ICIs response.

METHODS

C57BL/6 mice were intravenously (i.v.) injected with Lewis lung carcinoma (LLC1) cells and treated with aerosolized saline or vancomycin (50 mg)/neomycin (100 mg) 5 days/week. 16S rRNA gene metagenomics analysis and culturomics were performed on treated tumoral lungs and healthy lungs. Antibiotics aerosol was combined with intraperitoneal anti-PD-1 ICI (200 µg/mouse twice a week) and the effect evaluated by assessing the lung tumoral foci, in vitro lung immune cells cytotoxic activity and T cell subsets expansion by flow cytometry (FC) and single cell RNA (sc-RNA) sequencing.

RESULTS

Antibiotic aerosol was highly effective in eliminating many bacteria that had arisen specifically upon LLC1 tumor growth in lungs and resulted undetectable in healthy lungs, mostly belonging to Bacterioidales, Clostridiales and Enterobacteriales. This effect was associated with a significantly reduced number of lung tumoral foci, an increased cytotoxicity of infiltrating immune effector cells and a decrease of Tregs. Combination of antibiotics aerosol with anti PD-1 antibody improved ICI anti-tumor efficacy in mice i.v. injected with LLC1 tumor (p= 0,05) compared to anti PD-1 alone. Both Sc-RNA sequencing and FC revealed that this effect was associated with the expansion of stem cell memory (SCM) T cells in lung tumoral tissues. In vivo transfer by aerosol of cultivable Enterobacteriales strains isolated from tumor-bearing lungs, but not from healthy lungs, in lung of mice i.v. injected with LLC1 tumor cells reduced the anti-tumor activity of anti-PD1 antibody, suggesting a role of tumor-associated bacteria in restraining the response to ICI treatment.

CONCLUSIONS

These results reveal that signals imparted by the tumor-shaped microbiota impair the response to immunotherapy, and that the targeting of tumor-associated microbiota reduces the immunosuppression in the tumor microenvironment and boosts local immune T cell activation.

P085

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 B-cell chronic lymphocytic leukaemia (B-CLL), acute lymphoblastic leukaemia (ALL), and lymphoma, 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; B-CLL, n=47; lymphoma, 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 B-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 B-CLL comparing to lymphoma 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 lymphoma 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, B-CLL and lymphoma.

P086

COMPARATIVE ANALYSIS OF THE PERFORMANCE OF AUTOMATED DIGITAL CELL MORPHOLOGY ANALYZERS FOR LEUKOCYTE DIFFERENTIATION IN HEMATOLOGIC MALIGNANCIES: MINDRAY MC-80 VERSUS WEST MEDICAL HEMA VISION

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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 HemaVision (HV) and the gold standard, manual microscopy.

METHODS

75 patients (M: F 53:47%; median (min-max) age 63 ys (1-90)), with hematological malignancies (ALL= 4, B-CLL=20, AML=20, CML=5, lymphoma= 20, infection=6) were analyzed. Their smears were compared using the MC-80, HV, and manual microscopy. According to REF, the agreement between microscopy (reference method, REF), HV, and, MC80, was expressed as the median (IQR) of a given cell population/feature, with REF-HV 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-HV: 0.09 (-0.35 to 0.54); REF-MC80: 0.21 (-1.16 to 1.57)]; Ly% [REF: 45% (12.5-77.8); REF-HV: -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-HV: -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-HV: -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-HV: -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-HV: -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-HV: 0.18 (-0.16 to 0.51); REF-MC80: -4.10 (-5.81 to -2.40)]; metamyelocytes [REF: 0.00% (0.00-0.4); REF-HV: 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-HV: 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-HV: 32.55 (21.55 to 43.56), REF-MC80: 17.70 (10.18 to 25.23)]; smudge cells in CLL [REF: 64.8% (42.9-100.3); REF-HV: 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 HV. However, HV shows better agreement with microscopy than MC80.

P087

INDOOR AIR POLLUTION, INFLAMMATION AND PERIPHERAL BLOOD MONONUCLEAR CELLS MITOCHONDRIAL ACTIVITY

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BACKGROUND-AIM

According to the WHO, the entire global population is exposed to air pollution levels higher than recommended for health preservation. Ambient air pollution is a major health risk, leading to respiratory and cardiovascular mortality. The most important air pollutants are particulate matter (PM), nitric oxide (NO_2) and total Volatile Organic Compounds (tVOC). Pathophysiologic mechanisms through which air pollution triggers cardiovascular events include activation of oxidative stress/inflammation as well as the release of extracellular vesicles (EVs) which contribute to the local and systemic effects driven by air pollutants. Aim was to evaluate the effects of air pollutants on peripheral blood mononuclear cells (PBMCs) inflammatory response and mitochondrial activity.

METHODS

PBMCs were isolated from healthy volunteers; mitochondrial activity was assessed by Mitostress test; indoor pollutants were monitored by AirAssure Monitor; extracellular vesicles (EVs) were isolated from A549 human lung cells previously treated with PM (<4µm; EV^{PM}). EV^{PM} were injected in a model of zebrafish (Danio Rerio) and used to treat THP-1 monocytes.

RESULTS

7-day indoor exposure to air pollutants ($PM_{2.5}$, NO_2 and tVOC) reduced the mitochondrial functionality of PBMC isolated from 17 healthy volunteers (10 female) and, specifically, the increased exposure to NO_2 corresponded to a reduced mitochondrial spare respiratory capacity. This latter was negatively associated to circulating levels of the proinflammatory cytokine TNFα. To identify possible mediators of these systemic effects linked to air pollutants, EVs were isolated from A459 human lung cells treated with PM. The injection of EV^{PM} in zebrafish raised the expression of proinflammatory cytokines IL8 and IL1β. Moreover, when EV^{PM} were used to treat human PBMC, an increased gene expression of proinflammatory cytokines (IL1α, IL1β. IL6) was found, together with a reduced mitochondrial functionality.

CONCLUSIONS

Air pollutants favor, by means of EVs, a proinflammatory status, by reducing the mitochondrial activity and increasing the gene expression of proinflammatory cytokines in PBMCs. Future studies are necessary to demonstrate whether EVs could mediate further systemic effects of air pollutants in different cellular districts.

P088

TXNIP MEDIATES FOXO3A ANTICANCER ACTIVITY IN TAMOXIFEN RESISTANT BREAST CANCER

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BACKGROUND-AIM

Resistance to endocrine therapy is a significant obstacle in the treatment of estrogen receptor α positive (ER+) breast cancer (BC) patients. The transcription factor Forkhead box class O 3 (FoxO3a) is an oncosuppressor and seems to be a positive prognostic marker in ER+ and Tamoxifen resistance BC. Thioredoxin interacting protein (TXNIP) is a tumor suppressor gene with low expression levels in BC, and its downregulation is associated with a poor prognosis. Thus, the aim of this study is to assess TXNIP involvement in mediating FoxO3a antitumor activity in ER+ BC cells (BCCs) and in their Tamoxifen resistant (TamR) counterparts. In the attemp to identify new anticancer drugs, we also evaluated the effects of the anticonvulsant Lamotrigine (LTG) which induces FoxO3a expression, on TXNIP expression.

METHODS

ER+ MCF-7 and their TamR cells were employed as experimental models. Data from TamR cells were also confirmed in TamR/TetOn-AAA BCCs, a doxycycline (Dox) inducible system expressing the constitutively active FoxO3a gene. TamR metastatic cells from BC patients were used.

RESULTS

FoxO3a overexpression increased TXNIP mRNA and protein levels in Tam-sensitive and -resistant BCCs, while FoxO3a silencing led to their decrease. ChIP experiments showed a significant recruitment of FoxO3a to the TXNIP promoter region containing a Forkhead Responsive Element (FHRE) motif in BCCs, confirming FoxO3a role in regulating TXNIP gene expression. Moreover, TXNIP silencing abrogated FoxO3a inhibitory effects on cell proliferation, motility and invasiveness in TamR cells, highlighting TXNIP role in mediating FoxO3a activity. Additionaly, LTG inhibited proliferation and induced FoxO3a and TXNIP mRNA and protein expression in TamR cells derived from patients with metastatic breast cancer.

CONCLUSIONS

Our data demonstrate that TXNIP regulation is an additional mechanism through which the oncosuppressor FoxO3a inhibits TamR cells growth and progression. This reinforces the idea that FoxO3a could be a viable target for treating ER + tumours. In this context, the anticonvulsant LTG, for its ability to activate both FoxO3a and TXNIP oncosuppressors, might be considered as an adjuvant to standard endocrine therapy.

P089

IMPACT OF CIRCULATING EXTRACELLULAR VESICLES DERIVED FROM OBESE WOMEN ON NEOPLASTIC TRANSFORMATION OF NORMAL MAMMARY EPITHELIAL CELLS.

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BACKGROUND-AIM

The incidence of obesity has risen sharply worldwide, and growing evidence shows its impact on several malignancies, including breast cancer (BC). Excessive adiposity and high body mass index (BMI) significantly affect BC incidence, prognosis, and therapeutic response. Obese women exhibit larger tumor size, limph node involvement, metastatic spread, and poorer survival outcomes due to secretion of soluble factors. Aside from them, extracellular vesicles (EVs), nanoscale lipid-bilayer enclosed vesicles released by a variety of cells, are emerging as powerful regulators of cell-to-cell communication able to promote all BC stages. However, the impact of obesity-derived EVs in mediating protumoural transformation of breast epithelial cells has not been previously investigated.

METHODS

Circulating EVs were isolated from serum of normal weight (NW: BMI<24.9kg/m2) and overweight/obese (OW/Ob: BMI>25kg/m2) women, and characterized by Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA), and marker expression (Immunoblotting, IB). As human normal mammary epithelial cell line, MCF-10A cells were used. MCF-10A transformation was evaluated after acute or chronic treatment with EVs by proliferation, migration, invasion, qRTPCR assays.

RESULTS

Circulating EVs successfully extracted from the serum of NW and OW/Ob women were characterized. TEM and NTA showed oval or bowl-shaped EVs ranging between 50-200nm. Enrichment of EV markers HSP90/TSG101/CD81 were detected in EV isolated fractions. Elevated levels of circulating EVs were detected in OW/Ob women. Acute treatment of MCF-10A with OW/Ob EVs resulted in increased motility, invasiveness but it didn't establish a clear phenotype. In contrast, chronic exposure of MCF-10A cells to OW/Ob EVs significantly increased anchorage-independent cell growth, foci formation, motility, and invasion along with an up-regulation of epithelial-mesenchymal transition (EMT) markers.

CONCLUSIONS

Our preliminary data show a significant impact of circulating EVs during obesity on normal mammary epithelial cells. Given the rising prevalence of obesity, elucidating mechanisms by which circulating OW/Ob EVs may influence the malignant transformation of breast epithelial cells may provide specific targets for a more personalised management of obese BC patients.

P090

NEW INSIGHTS IN PANCREATIC CANCER DEVELOPMENT: A NOVEL TUMORIGENIC ROLE FOR JAGGED1 IN PDAC TUMOUR.

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BACKGROUND-AIM

Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant tumor characterized by a poor prognosis and rapid progression. Activating mutations affecting Ras are the most common oncogenic events in PDAC, triggering multiple downstream pathways, including MAPK signalling cascade. Jagged1 ligand is a single-pass transmembrane protein that transactivates Notch receptors through cell-cell contact. Our recent works have shown that Jagged1 is processed by K-Ras-induced sequential proteolytic cleavages, culminating in the release of the Jagged1 intracellular domain (Jag1-ICD), which plays a significant role in colorectal cancer progression, gaining oncogenic events. Based on these reports, we aim to study the Jag1-ICD role in sustaining PDAC progression.

METHODS

SiRNA-mediated depletion of K-Ras, Notch1 e Jagged1 in PDAC cell lines; pharmacological treatments by using MEK/Erk inhibitors; generation of Jagged1-depleted by CRISPR/Cas9 technology and stable overexpressing Jag-ICD PDAC cell lines; proliferation and invasion assays. In vivo xenotransplantation experiments in mice.

RESULTS

Our results highlight a direct relationship between K-Ras signature with Notch1 and Jagged1 deregulation in PDAC. Indeed, K-Ras silencing in several PDAC cell lines induces a marked impairment of Notch1 activation, significantly decreasing Jagged1 expression and Jag1-ICD processing. These observations suggest a role for K-Ras/Erk signalling in controlling Jag1-ICD expression/activation via Notch pathway. Our data indicates the existence of a K- Ras/ERK/Notch1/Jagged1 signalling axis, ultimately triggering a strong release of Jag1-ICD that moves into the nucleus and gains oncogenic function. In accordance, we observed that Jag1-ICD nuclear accumulation can modulate proliferative and EMT events in vitro and in vivo experiments, suggesting a tumorigenic role of Jag1-ICD in PDAC.

CONCLUSIONS

Our observations indicate that Jagged1 may act as a novel oncogenic driver in pancreatic cancer by directly/indirectly interacting with other dysregulated pathways, such as the K-Ras/MEK/Erk axis. The study of this novel oncogenic pathway may provide an opportunity to gain new insights into pancreatic cancer development and address new potential therapeutic approaches.

P091

DNA METHYLATION-DERIVED BIOLOGICAL AGE AND LONG-TERM MORTALITY RISK IN SUBJECTS WITH TYPE 2 DIABETES

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BACKGROUND-AIM

Individuals with type 2 diabetes (T2D) have an elevated mortality risk that traditional risk factors do not fully explain. Estimating biological age via DNA methylation (DNAm)-based biomarkers, often referred to as "epigenetic clocks", is a promising method for enhancing risk assessment for various outcomes. This study investigates whether DNAm-derived biological age can predict mortality in T2D patients independently of common risk factors.

METHODS

Using a well-characterized cohort of 568 T2D patients followed for 16.8 years, we conducted a genome-wide DNAm analysis on peripheral blood leukocytes from deceased (n=28) and survived (n=28) subjects matched by propensity scores. We analyzed DNAm with the Infinium Human MethylationEPIC BeadChip (Illumina) to determine biological aging using validated epigenetic clocks and assessed DNAm-estimated levels of specific inflammatory proteins and blood cell counts. We tested the associations of these estimates with mortality using two-stage residual-outcome regression analysis, establishing a reference model based on data from the survivors.

RESULTS

Deceased subjects had a higher median epigenetic age according to the DNAmPhenoAge algorithm (57.49 [54.72; 60.58] years vs. 53.40 [49.73; 56.75] years; p=0.012) and an accelerated pace of aging measured by the DunedinPoAm (1.05 [1.02; 1.11] vs. 1.02 [0.98; 1.06]; p=0.012). DNAm PhenoAge was associated with mortality independently of traditional risk factors (HR 1.16, 95% CI 1.05-1.28; p=0.004). Epigenetic indicators of three chronic inflammation-related proteins (CXCL10, CXCL11, C-reactive protein, and enRAGE) and DNAm-based estimates of exhausted CD8+ T cell counts were higher in deceased subjects compared to survivors.

CONCLUSIONS

Our findings suggest that DNAm-derived biological age and associated epigenetic markers are potent predictors of mortality in T2D patients, independent of traditional risk factors. This study supports the integration of DNAm-based tools in improving mortality risk stratification and highlights the potential for incorporating DNAm biomarkers in monitoring and managing T2D progression and complications. Further research is required to validate these findings and explore the clinical applications of DNAm biomarkers in T2D.

P092

DIFFERENTIAL REGULATION OF WINGLESS-WNT/C-JUN N-TERMINAL KINASE CROSS-TALK VIA OXIDATIVE EUSTRESS IN PRIMARY AND METASTATIC COLORECTAL CANCER CELLS

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BACKGROUND-AIM

In tumor microenvironment (TME) ROS production affects colorectal cancer (CRC) survival, progression and therapy resistance. H2O2-mediated oxidative stress can modulate Wnt/β-catenin signaling and TME metabolic reprogramming. Currently, it is unclear how mild/moderate oxidative stress (eustress) modulates Wnt/β-catenin and JNK signaling relationships in primary and metastatic CRC cells.

METHODS

We analyzed the molecular link between oxidative distress and Wnt/β-catenin/JNK in APC mutated primary SW480 and metastatic SW620 CRC cells treated with JNK inhibitor SP600125 and H2O2 0.005 mM to 1 mM. We assessed cell viability, mitochondrial respiration, glycolysis, and Wnt/β-Catenin/JNK gene and protein expression.

RESULTS

Increasing H2O2 concentrations modulated mitochondrial viability without significantly reducing it, up to 0.05 mM, with differential effects between primary and metastatic tumor cells. After JNK inhibition and low dose H2O2, both cell lines showed a slightly reduced mitochondrial activity, while the oxygen consumption rate decreased in the metastatic model. In primary CRC cells, both H2O2 alone and SP600125 significantly increased APC, LEF1, LRP6, JUN/AP1, cMYC and IL8 ex-pression, whereas in metastatic CRC cells this effect occurred after JNK inhibition. The results showed differential cross-regulation of Wnt/JNK in primary and metastatic tumor cells under environmental eustress conditions.

CONCLUSIONS

We suggest that H2O2-mediated oxidative stress may also modulate the canonical and non-canonical Wnt/β-catenin signaling pathways in CRC cells linked to the JNK pathway and metabolic program of TME. Understanding how primary and metastatic CRC cells adapt to a H2O2-producing in-testinal microenvironment might provide new insights for therapeutic interventions.

P093

PATHOPHYSIOLOGICAL ASPECTS OF LIVER DAMAGE IN PREGNANT RATS UNDER EXPOSURE TO ADVERSE EXOGENOUS FACTORS

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BACKGROUND-AIM

According to the WHO data there is an increase in number of gastro-intestinal tract pathology cases. One of the most significant parts of these cases is liver damage. A key role in the development damage in mother's liver during pregnancy belongs to different exogenous adverse factors of healthy life-style such as pernicious habits, unbalanced nutrition etc.The aim of present work is to determine the aspects of liver damage among pregnant rats under exposure to adverse exogenous factors.

METHODS

The research was conducted 27 rats (WAG population). Rats were subdivided into 3 groups: 1) the control group; 2) rats affected by hypodynamic stress; 3) rats underwent the unbalanced nutrition. The complex of morphometric and biochemical analysis of liver tissue was carried out to evaluate the rate of liver damage from factors mentioned above.

RESULTS

Generally homotypic changes in liver structure were observed both after chronic stress and excessive nutrition. The dyscompletion of acini structure, the increase of binucleate cells quantity were observed in both experimental groups, however with the difference in rate (120% in 2nd group, 50% in the 3rd one). Moreover, the stromal-parenchymal index was increased because of relative stroma volume growth among the rats of 2nd group. At the same time the examination of liver homogenate samples revealed the similarity of changes in tissues of 2nd and 3rd rat groups including the rise of cholesterol, non-etherified fatty acids and triglycerides together with differently rated lowering of phospholipids.

CONCLUSIONS

The mechanisms of chronic stress impact which may lead to liver cirrhosis and fibrosis appeared to be almost identical to the effects of excessive nutrition.

P094

EVIDENCE THAT Y537S MUTANT BC CELLS MAY CONVERT NFS INTO CAFS IN TUMOR MICROENVIROMENT

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BACKGROUND-AIM

It is widely demonstrated how endocrine therapy (ET) has significantly improved the clinical outcomes of Estrogen receptor alpha-positive (ERα+) breast cancer (BC) patients, even though the resistance to ET remains a frequent event to be faced. Mutations in the hormone-binding domain of ERα are hot spot mutations in ER+ BC patients who developed resistance to ET. On the other hand, several evidence highlighted how resistance to ET may depend other than by the intrinsic mutations of ERα, by their interaction with tumor microenvironment (TME). In the present study, we investigated how the intrinsic malignancy of mutant BC cells may influence the behavior of the fibroblasts.

METHODS

To this aim we conducted co-culture experiments of fibroblasts with mutant BC cells obtained from MCF-7 cell lines engineered using CRISPR-Cas9 encoding the ESR1 mutation Y537S with either normal fibroblasts (NFs) or cancer associated fibroblasts (CAFs) isolated from human BC specimens. Label-free liquid chromatography-tandem mass spectrometry and gene ontology analysis using Metacore software were performed to investigate how functional interactions between Y537S and fibroblasts may affect their proteomic profile.

RESULTS

We found that the exposure to YS1 conditioned medium (CM), exhibiting a higher amount of insulin growth factor-1 (IGF-1), affected cell morphology, proliferation and migration of NFs and CAFs. It was very interesting to observe that NFs exposed to YS1-CM began to acquire a proteomic profile similar to CAFs. Among the 113 up-regulated proteins shared between NFs/CM-YS1 and CAFs we found that Yes-associated protein 1 (YAP1) is the main central hub in the direct interaction network analyzed by Metacore software. The enhanced YAP1 mRNA levels, protein expression and activity induced by the exposure to YS1-CM is no longer noticeable in the presence of IGF-1 immunodepletion.

CONCLUSIONS

From our data, it emerges that YS1 involving also IGF-1 signaling display a specific role in the conversion of NFs into CAFs through the activation of YAP1 pathway as the main regulator of fibroblast stretching, cytoskeleton adaptation, spread cell shape and cell motility.

P095

THE ROLE OF PATHOPHYSIOLOGY IN MODERN MEDICAL EDUCATION

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BACKGROUND-AIM

Understanding the development mechanism of any disease is essential for treatment conduction. It is important to define the etiological features, triggers of pathogenesis and vicious circle conditions. Another key point is the forming the clinical thinking patterns of future doctors. The aim of this work is to define the role of pathophysiological studies in higher medical education.

METHODS

39 students of 3 – 6 courses from I – III medical faculties of Kharkov National Medical University were involved into sociological survey. Quiz was distributed in the most popular social networks .

RESULTS

The analysis of data obtained from survey showed some remarkable results. 95% of respondents considered the understanding of pathologies and diseases development is crucial for correct diagnostics and treatment, and the rest 5% could not answer this question. But at the same time only 68% of respondents mentioned that exactly pathophysiology helped them to cope with certain clinical case. Also 32% reported that knowledge in pathophysiology field compelled them to change their views on many doubtful or pseudoscientific theories. Together with that 89% noted the induction of interest to this discipline was inspired by teacher during study, 11% said that this interest had been present long before they entered the exact department. 48% of respondents stated that study of pathophysiology discipline motivated them to receive further education; another 50% noted the inspiration for scientific research and only 2% reported about no significant influence of pathophysiological studies on them.

CONCLUSIONS

Based on the data obtained it can be concluded that majority of students objectively estimate the significance of pathophysiology discipline for their future professional activity, and it was also showed the especial importance of lecturer in mentioned estimation.

P096

CBX6 ROLE IN GLIOBLASTOMA MULTIFORME

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BACKGROUND-AIM

Chromobox (CBX) family consists of eight important epigenetic regulatory proteins, highly conserved in both mouse and human genomes.CBX members contain a chromobox domain able to interpret the two principal gene suppressive epigenetic marks H3K9me3 and H3K27me3[PMID:32979540].CBXs are essential regulators of various biological processes, such as embryonic development, stem cell self-renewal, cell proliferation, apoptosis and lineage-commitment[PMID:32979540]. Some CBX family proteins are canonical components in Polycomb repressive complex 1(PRC1) contributing to the complex gene transcriptional repression mechanism[PMID:24793759].CBX family proteins are involved in the occurrence and progression of different cancer types[PMID:35210369], including glioblastoma multiforme (GBM)[PMID:24260522]. CBX6 oncogenic role has been demonstrated in hepatocellular carcinoma[PMID:28122351], bladder cancer[PMID:37189494] and breast cancer [PMID: 30655550].GBM is the most common aggressive and deadly malignant primary brain tumors[PMID:38505467].Project aim is CBX6 molecular and functional characterization as prognostic marker and therapeutic target in GBM

METHODS

Cell Culture;Viral Transfection;RT-PCR;WB;IF;NGS data analysis;Genome editing

RESULTS

We revealed a significant downregulation of CBX6 expression in GBM primary samples (N°163), compared to normal tissues (N°207), performing NGS data analysis from TCGA.The results showed significant negative correlation between CBX6 expression and IDH mutation, known predictive marker of GBM favourable outcome.Experimental data verified the reduction in CBX6 expression, at protein and gene level, in GBM cell lines (U87 and U138) compared to fetal glial cells(SVG P12).Interestingly,we checked the CBX6 increase in U87 (IDH1 R132H Mutated) compared to U87 WT. Furthermore we characterized CBX6 exclusive nuclear localization by IF and WB.We investigated CBX6 genomic profile analyzing different histone marks using ChIP-seq and chromatin accessibility data by ATACseq.Moreover,we identified some CBX6 gene targets, transcriptionally regulated by direct bond on their promoter region; many of these targets are involved in biological processes significant in the brain and GBM development.We, also,revealed a transcriptional downregulation of some CBX6 interactors in PRC1 complex (RNF2, PCGF2 and PHC1 in U87;RNF2, PHC2, PHC3, PCGf2 and PHC1 in U138) and all PRC2 components, as compared to SVGP12

CONCLUSIONS

CBX6 seems a promising prognostic/diagnostic marker and therapeutic target in GBM

P097

CIRCULATING EV-MICRORNAS IN METASTATIC MELANOMA AS NON-INVASIVE BIOMARKERS FOR DIAGNOSIS AND TREATMENT RESPONSE

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BACKGROUND-AIM

Melanoma is the most aggressive skin cancer, with 50% of patients with metastatic or unresectable melanoma facing high 5-year mortality. Identifying non-invasive biomarkers for diagnosing and stratifying metastatic melanoma could prove essential to guide treatment decisions. MicroRNAs (miRNAs), small non-coding RNA molecules abundant in extracellular vesicles (EVs), have been studied as biomarkers in various cancers. This study aims to identify EV-miRNAs as circulating biomarkers in metastatic melanoma and assess their role in treatment response.

METHODS

We explored EV-miRNAs in unresectable stage III and IV melanoma patients treated with immune checkpoint inhibitors (ICI) and Guadecitabine (NIBIT-M4 clinical trial). Plasma was collected before treatment (W0) and 12 weeks (W12) after treatment, and EVs were isolated for miRNA profiling. EV-miRNA profiling was first conducted on W0 samples and on a matched cohort of healthy donors.
Enrichment analysis and logistic regression analysis were used to characterize differentially expressed (DE) EV-miRNAs at W0 and identify a specific signature distinguishing metastatic melanoma patients from healthy individuals. This signature was validated in an external publicly available dataset and in an independent internal cohort using droplet digital PCR, confirming its diagnostic relevance.
Clinicians classified patients as responders (R) or non-responders (NR), and EV-miRNA profiles at W12 were analysed to be associated with treatment response.

RESULTS

At W0, EV-miRNAs profiling found 65 DE EV-miRNAs distinguishing melanoma patients from healthy controls. The enrichment analysis performed on these DE EV-miRNAs showed their involvement in migration, angiogenesis, and immune regulation. Logistic regression analysis identified as a signature 4 EV-miRNAs (3 up-regulated: miR-412-3p, miR-507, miR-1203; 1 down-regulated: miR-362-3p) distinguishing patients from healthy controls.
At W12, 58 EV-miRNAs were modulated compared to W0, and two EV-miRNAs, including one from the diagnostic signature, were associated with treatment response.

CONCLUSIONS

These findings suggest that the two discovered EV-miRNAs could be useful as non-invasive biomarkers for monitoring response to ICI and Guadecitabine treatment, highlighting EV-miRNAs as a tool for precision medicine.

P098

STUDY OF CIRCULATING MICRORNAS AS BIOMARKERS FOR GUIDED IMMUNOTHERAPY IN HEAD AND NECK CARCINOMA PATIENTS: A TOOL FOR PERSONALIZED MEDICINE

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BACKGROUND-AIM

Head and neck squamous cell carcinomas (HNSCC) are solid tumors originating from the mucosa of the upper aerodigestive tract, pharynx, larynx, mouth, and nasal cavity. HNSCC is the sixth most common cancer worldwide characterized by metastatic evolution and poor prognosis. Tobacco, alcohol and human papillomavirus represent major risk factors. Immune checkpoint blockers (ICBs) for advanced HNSCC stages have been recently approved as standard therapy. Effectiveness of immunotherapy is heterogeneous and varies among patients and HNSCC types, which highlights the need to identify reliable biomarkers able to predict response to therapy. MicroRNAs (miRNAs) are a class of small non-coding RNAs involved in the regulation of gene expression. They are detectable in cells and body fluids such as blood, and in the latter, they are ideal candidates as non-invasive biomarkers since they demonstrate dynamic changes related to disease status.

METHODS

Nanostring nCounter Human v3 miRNA expression assay (NanoString Technologies, Seattle, WA, USA) was used to determine the miRNAs expression profile from plasma samples of a discovery cohort of HNSCC patients treated at first line with immunotherapy and from a cohort of healthy controls. The analysis of raw miRNA data was performed using nSolver 4.0 Software (NanoString, Seattle, WA, USA). Before data normalization, nCounter data quality control (QC) was assessed.

RESULTS

Nanostring profiling of 800 miRNAs resulted in a clear separation of patients and healthy controls. Differential expression analysis between Responder (R) and Non-responder (NR) patients to immunotherapy treatment allowed the identification of 21 miRNAs differentially expressed, of which 3 microRNAs upregulated and 18 microRNAs downregulated in NR.

CONCLUSIONS

Taken together, these data report a series of miRNAs that could be used as predictive biomarkers of response to immunotherapy treatment. Ongoing experiments are focused on the validation of the differentially expressed miRNAs in an independent patients' cohort and in shedding light to their biological role.

P099

ERK5 INHIBITION MODULATES EGFR EXPRESSION AND LOCALIZATION GENERATING NEW TARGETING OPPORTUNITIES IN HEPATOCELLULAR CARCINOMA

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BACKGROUND-AIM

Hepatocellular Carcinoma (HCC) is the most common form of liver cancer and accounts for 90% of cases. Nonetheless, available molecular-targeted therapies are still unsatisfactory so that additional therapeutic targets need to be identified. The extracellular signal-regulated kinase 5 (ERK5) is a member of the Mitogen-Activated Protein Kinases (MAPK) family, and is highly expressed in hepatocytes, and its gene has been reported to be amplified in HCC. Moreover, we recently reported that ERK5 regulates the development and growth of HCC, so that ERK5 targeting appears as a promising approach for the treatment of this cancer.

METHODS

We used the human hepatocellular carcinoma cell lines Huh-7 and HepG2. Gene silencing was performed with short harpin RNA for ERK5. For the pharmacological treatments, ERK5 (JWG-071) and nuclear import protein (ivermectin) inhibitors effects were evaluated after 72h of treatment in term of cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The mRNA expression was analyzed using quantitative RT-PCR. Protein expression levels and intracellular localization were detected by Western blot and immunofluorescence in confocal microscopy.

RESULTS

We found that ERK5 knock down (KD) increased EGFR mRNA and protein levels, and downstream PI3K/AKT activation. In addition, the amount of nuclear EGFR was higher in ERK5-KD HCC cells compared to control cells. Interestingly, the combination of ERK5i and nuclear translocation inhibitor ivermectin resulted to be more effective than single treatments in reducing cell viability in both cell lines.

CONCLUSIONS

Combined treatments targeting ERK5 and EGFR nuclear translocation could provide an effective additional therapeutic strategy for hepatocellular carcinoma.

P100

IMMUNOPHENOTYPIC CHARACTERIZATION OF THE CELL INFILTRATES IN CHRONIC SUBDURAL HEMATOMA: A PILOT STUDY ON NEW PATHOPHYSIOLOGICAL AND TRANSLATIONAL ASPECTS

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BACKGROUND-AIM

Chronic subdural hematoma (CSDH) is an encapsulated collection of
fluid, blood, and blood degradation products layered between the arachnoid and dura
mater, remaining one of the major neurosurgical emergencies. In this regard, it is urgent to find biomarkers capable of predicting patients at the highest risk of recurrence.

METHODS

This pilot study involved 5 patients with CSDH admitted to the Neurosurgery Department of the University Hospital of Salerno and 3 healthy donors for comparison. During surgery, peripheral blood and hematoma fluid samples were collected in sterile vacutainer tubes containing EDTA and/or heparin. To characterize the immune infiltrate in the three compartments (subdural fluid, patients' peripheral blood, and healthy volunteers' peripheral blood), mononuclear cells were extracted from the respective fluids by density gradient centrifugation using Ficoll. The cells were stained with a mix of fluorochrome conjugated
antibodies against human CD66-CD45-CD56-CD19-CX3CR1-CD3-CD16-CD14-CD31 and CD8
to characterize the immunophenotype by FACS analysis.

RESULTS

The average age of the patients was 66 years, with an average GCS score of 13.4 at admission.
The immunophenotypic analysis of the individual compartments showed that the CSDH infiltrate consists of an adaptive immunity cell population, predominantly T lymphocytes, with 22%±11.59% being cytotoxic T lymphocytes, and a population of B lymphocytes making up 6.81%±6.3%. The innate immunity cell populations isolated in the three compartments showed few NK cells (0.65% ± 0.34%), but surprisingly many NK-T cells, which were 6.58%±4.1%. Monocytes accounted for 5.8% ± 0.8%.
For the first time, an erythro-myeloid progenitor population (CD45+CD31+) was identified. Statistical analysis revealed that CD45lowCD31low cells were significantly absent in the peripheral blood of healthy controls compared to patients, and their value positively correlated with peripheral platelet count (p=0.009). NK cells in CSDH patients were significantly lower in both central and peripheral compartments compared to healthy controls Conversely, NK-T cells were higher in both central and peripheral compartments of CSDH patients compared to healthy controls.

CONCLUSIONS

This study provides for the first time the nature of the cellular composition of the leukocyte infiltrate in CSDH, with novel pathophysiological insights, and provides preliminary indications for the definition
of a biomarker that correlates with clinical outcomes

P101

INVOLVEMENT OF MALARIA PIGMENT IN MACROPHAGE POLARIZATION: AN IN VITRO MODEL OF PRIMARY HUMAN MONOCYTE-DERIVED MACROPHAGES

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BACKGROUND-AIM

Malaria is a vector-borne disease, caused by Plasmodium protozoan parasites. Hemozoin (HZ) or malaria pigment is the detoxification product of heme, produced by parasites during their intra-erythrocytic stages following hemoglobin digestion. During the asexual replication, HZ is released in the bloodstream and ingested by phagocytic cells, especially macrophages, inducing different, often opposite immune responses. Macrophages can be polarized to a spectrum of intermediate phenotypes between two extreme subsets: M1 (pro-inflammatory) and M2 (anti-inflammatory, tissue-remodelling).
The aim of this project is to unravel the effects of HZ in an in vitro model of primary human monocytes-derived macrophages

METHODS

Primary human monocyte-derived macrophages (MDM) were differentiated from peripheral blood monocytes of healthy donors and polarized towards M1 pro-inflammatory phenotype (IFNγ+TNFα), M2 anti-inflammatory phenotype (IL-4) or left unpolarized (M0) in presence or not of HZ from P. falciparum cultures. After 24h of incubation, mRNA levels of TNFA and IL1B or PPARG, genes typically modulated in M1 or M2 macrophages, respectively, were evaluated by Real-Time PCR; IL-1ß, IL-10, IL-6, CXCL8 concentrations in supernatants were quantified by ELISA

RESULTS

HZ induced significantly higher levels of TNFA mRNA in M0. A slight, non-significant increase of TNFA mRNA was also observed in both M1 and M2 phenotypes. HZ induced significantly higher levels of IL1B mRNA in all the tested conditions (M0, M1, M2) suggesting a pro-inflammatory effect of malaria pigment independent from the initial inflammatory state of macrophages. Conversely, PPARG mRNA levels were not modulated by HZ. CXCL8 secretion was increased by HZ independently from macrophage polarization. IL-1ß, IL-10, IL-6 were not detectable by ELISA

CONCLUSIONS

These results confirm that HZ is a pro-inflammatory stimulus in unpolarised macrophages. For the first time the effect of HZ in the presence of M1 or M2 stimuli was evaluated, suggesting a role in modifying macrophage plasticity towards a pro-inflammatory state

P102

IN VITRO MODEL OF LIVER ISCHEMIA/REPERFUSION INJURY IDENTIFIES IRHOM2 AS A NEW TARGET OF DISEASE

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BACKGROUND-AIM

Ischemia-reperfusion injury (IRI) is one of the most important causes of liver organ rejection in acute and chronic phases. IRI generates an inflammatory environment by activating resident Kupffer cells, leading to compromised allograft functionality and rejection. In the last ten years, iRhom2 emerged as an essential regulator of tumor necrosis factor α (TNFα) release by controlling the activity of TNFα converting enzyme (TACE), thus implying its involvement in several inflammatory diseases. For these reasons, our goal was to investigate iRhom2 function in IRI since no data have been described yet on its potential role at the onset of the injury.

METHODS

In vitro IRI was tested on primary macrophages and iPSCs-derived hepatocytes by using single or co-culture approaches. In vitro cold ischemia was performed with 2% O2 exposition in a preservation medium. Warm reperfusion was performed at 1, 4 and 24 hrs. An array of molecular, cellular, and -omic assays was used to evaluate iRhom2 role during IRI.

RESULTS

We demonstrate that iRhom2 is involved in liver IRI progression in transplanted patients, and its downregulation in M1-like primary macrophages modulates the secretion of pro-inflammatory cytokines and danger-associated molecular patterns (DAMPs) associated with IRI damage. Interestingly, iRhom2 silencing accelerates macrophage recovery during reperfusion and acts in IRI damage in a TACE-independent fashion, since TACE inhibition has little effect on cytokine release. Finally, iRhom2 activity induces an IRI-associated senescence phenotype on injured iPSC-derived hepatocytes by secreting a cocktail of cytokines and DAMPs that promote it via activation of p21/p53 signaling pathway on the target cells. Notably, we demonstrated that the senescence phenotype can be rescued by using anti-HMGB1 neutralization antibodies and not with anti-TNFα ones, suggesting that HMGB1 is required to induce hepatocytes senescence, and further sustaining the hypothesis that iRhom2 in IRI acts in a TACE-independent fashion.

CONCLUSIONS

We found that iRhom2, in response to IRI, could regulate the secretion of cytokines and DAMPs responsible for the progression of the damage, by a mechanism that might be independent of TNF pathway. Thus, iRhom2 inhibition during IRI might represent a promising target to improve recovery from IRI damage and may prevent hepatocyte senescence.

P103

SIMULTANEOUS INHIBITION OF EGFR, AXL AND FAK/WNT SIGNALING PATHWAYS WITH DRUG COMBINATIONS IN MALIGNANT MESOTHELIOMA

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BACKGROUND-AIM

Malignant mesothelioma (MM) is an aggressive tumor with a poor prognosis. Abnormally activated pathways in MM cells include those activated by EGFR/ErbB2. EGFR tyrosine kinase inhibitors (TKI) are used to treat MM patients. Clinical studies have shown the occurrence of resistance to TKI in MM patients, due to the activation of alternative pathways, including those activated by Axl and Wnt. Thus, simultaneous treatment of MM with drugs targeting different signaling pathways might be a promising therapeutic approach. AFA is an ErbB receptors-family blocker that covalently binds to the kinase domain of EGFR, ErbB2, ErbB4. TP-0903 (TP) selectively inhibits Axl, a receptor of the TAM family able to activate the MAPK, PI3K/Akt, JAK/STAT, NF-κB pathways. Y15 (Y) is a small molecule that directly inhibits Y397 autophosphorylation of FAK, which plays a key role in controlling cancer migration and EMT. We evaluated a novel therapeutic drug combination for the treatment of MM by employing inhibitors of the EGFR (AFA), Axl (TP) and FAK/Wnt (Y) pathways to overcome resistance to EGFR-TKI and to enhance the drug's effects on tumors.

METHODS

The effects of AFA, Y and TP, alone or in combination, on cell proliferation, apoptosis, and modulation of pro-survival signaling pathways were evaluated in cultured human and mouse MM cell lines of different histotypes. In addition, tumor spheroids from MM cells were generated and then treated with the drugs.

RESULTS

As assessed by SRB and Trypan blue assays, double (AFA+TP and AFA+Y) and triple (AFA+TP+Y) treatments enhanced the effect of AFA on MM cells. The triple combination of drugs induced a stronger antitumor effect, resulting in increased growth inhibition and cell death. The single and combined treatments were able to affect EGFR, Axl and FAK/Wnt pathways. In addition, lower doses of the single inhibitors significantly reduced spheroid diameter, with even more pronounced results when used in dual combinations. Finally, the triple combination was the most effective in reducing tumor spheroid growth compared to the same drugs used alone or in double combinations.

CONCLUSIONS

These results may lead to the identification of new combination therapeutic strategies for MM treatment.

P104

ANTITUMOR EFFECT OF COMBINATION OF ERBB RECEPTORS, AXL AND FAK INHIBITORS IN 2D AND 3D HEAD AND NECK CANCER MODELS

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BACKGROUND-AIM

The Head-Neck Cancer (HNC) represents a heterogeneous group of neoplasms, which differ from each other in terms of histological, molecular and clinical perspectives. The standard therapy for HNC patients is a combined approach of surgery, radio- and chemotherapy. However, the prognosis of patients remains poor. Thus, it is necessary to develop new therapeutic strategies. The aim of this study is to determine the combined effect of different molecular target drugs, such as Afatinib (AFA; ErbB inhibitor), TP-0903 (Axl inhibitor) and Y15 (FAK inhibitor), used at low doses, in inhibiting HNC growth.

METHODS

Human HNC cell line isolated from the tongue (Cal-27), pharynx (FaDu) and salivary gland (A-253) were used to evaluate the antitumoral effects of AFA, TP-0903 and Y15, alone or in combination. Cell proliferation, death and migration were evaluated with Sulforhodamine B, Trypan Blue and Wound Healing assay, respectively. The effect of combined drugs on proliferation and apoptosis were also evaluated in HNC spheroids. Western blotting analysis were performed to investigate the expression and phosphorylation of several proteins involved in signaling transduction or in the epithelial to mesenchymal transition.

RESULTS

This study showed that the combined treatment of AFA, TP-0903 and Y15, used at low doses, was able to significantly inhibit tumor growth, to increase cell death and to reduce the migration of HNC cells as compared to drugs used alone or in double combination. In addition, the simultaneous combination of the three inhibitors significantly reduced the growth of A253 tumor spheroids, and increased apoptosis. Moreover, the combined treatment was more effective than the single one in decreasing phospho-ERK levels as well as the phosphorylated levels of the pro-survival kinase AKT in all cell lines.

CONCLUSIONS

Our study shows that combined treatment with three different signaling pathways inhibitors significantly inhibits the growth and tumor progression of HNCs. In addition, the use of 3D cultures is optimal for studying these inhibitors on more complex systems and thus more representative of in vivo tumors. Therefore, this study may have new clinical implications for the development of novel therapeutic approaches for HNC.

P105

EFFECTS OF ADMINISTRATION OF BISPHENOL-A IN DRINKING WATER IN MALE MICE TRANSGENIC FOR THE NEU ONCOGENE, WHICH SPONTANEOUSLY DEVELOP SALIVARY GLAND ADENOCARCINOMA

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BACKGROUND-AIM

Head and neck cancer (HNC) is the seventh leading cause of cancer-related morbidity and mortality. 6-8% of HNC are salivary gland carcinomas. Bisphenol-A (BPA) is used in the production of plastics and plastic-derived products, and it could be transferred in small amounts into food and drink. BPA acts as an endocrine disruptor by binding to the estrogen receptor (ER). Thus, the oral cavity may be the primary source of BPA contamination because this chemical can interact with ER, which are found in keratinocytes and oral epithelium.
The aim of this study is to define the molecular mechanisms of action of BPA in genetically modified mouse models of adenocarcinoma of the salivary gland, to assess its contribution in the carcinogenesis and progression of this cancer.

METHODS

BPA was administered to male transgenic BALB-neuT mice in drinking water. These mice expressed the activated HER2/neu oncogene, which caused a slow-growing, multifocal acinic cell adenocarcinoma that involves the parotid and then the submandibular glands. The in vivo effects of BPA in mice were studied by monitoring tumor growth and by analyzing the effect on the expression of several markers in tumor tissues. Furthermore, to understand the mechanisms underlying the pro-tumoral effects of BPA, the in vitro effects of BPA treatment on cell proliferation, cell death and modulation of the expression of proteins involved in cell growth were evaluated in murine (SALTO-5) and human (A-253) salivary gland cancer cell lines. SALTO-5 cell line derives from the BALB-neuT transgenic male mice.

RESULTS

BPA in drinking water accelerated the ErbB2/neu-mediated neoplastic transformation of salivary glands in the transgenic male BALB-neuT mice and reduced the animal survival. Histological characterization of tumor tissues showed that BPA exerted a pro-tumoral effect by affecting the expression of cell proliferation (Ki67, phospho-AKT), neo-angiogenesis and invasiveness markers.
Our in vitro findings demonstrate that BPA induced SALTO-5 and A-253 cancer cells proliferation after chronic, low-dose exposure.

CONCLUSIONS

Our data indicate that administration of BPA induced neoplastic transformation of salivary glands earlier through stimulation of cell proliferation in the BALB-neuT mouse model.

P106

NTF RESCUE RETINA DEGENERATION IN DBA/2J MICE

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BACKGROUND-AIM

Glaucoma is an optic neuropathy characterized by a progressive degeneration of retinal ganglion cells (RGCs) and gradual loss of visual function. Elevated intraocular pressure (IOP) is widely recognized as the major risk-factor of neuronal injury as well as the only modifiable determinant. Currently, lowering IOP is the main therapeutic approach for slowing disease progression in patients with glaucoma. However, this strategy is insufficient for preventing disease progression. Several studies have suggested that glaucoma is driven by interconnected pathogenic processes that comprise biomechanical, vascular, metabolic, oxidative and inflammatory components. Analogies with neurodegenerative pathologies correlated to inflammatory responses justify the recent focus on targeting retinal neuroinflammation and preserving RGCs. 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 once intravitreally. Electroretinogram (ERG) and Optometry tests have been performed before compound administration and 4 weeks after treatment . At the end of the study, IHC and western blot assays on enucleated eye samples were used for molecular studies.

RESULTS

No mortality as well as no pathological signs were observed during the study 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 at 0.1, 1 and 10 cd s/m2 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 IBA-1 and GFAP expression as well as a widely reproducible reduction of proinflammatory cytokines (IL1b, IL6, and TNFa) and apoptosis (cleaved PARP) after treatment with NTF.

CONCLUSIONS

Taken together, our results confirm the efficacy of the NTF in improving the visual acuity and rescuing the electrophysiological impairment of retina induced by glaucoma. Furthermore, our data demonstrate a deep impact of this molecule in reducing glaucoma-related neuroinflammation

P107

IN VIVO EFFECTS OF DAILY ORAL INTAKE OF THE ENDOCRINE DISRUPTOR CADMIUM (CD) IN MICE TRANSGENIC FOR THE NEU ONCOGENE, WHICH SPONTANEOUSLY DEVELOP MAMMARY TUMORS

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BACKGROUND-AIM

Breast cancer represents the most prevalent cancer worldwide. Environmental pollutants can increase cancer risk and modulate cancer aggressiveness by acting as endocrine disruptors (EDs). Food and environmental contamination are the primary sources of Cadmium (Cd) exposure for the general population. Cd is used in various industrial applications, including batteries, pigments, plastic stabilizers, and corrosion resistance compounds. Cd displays ED properties due to its ability to bind estrogen receptors (ER) and act as a xenoestrogen. It can cause oxidative stress leading to DNA damage, and alter the expression of genes involved in cell growth, apoptosis, and metastasis. In the present study, we evaluated Cd's contribution to cancerogenesis in mice transgenic for the neu oncogene (BALB-neuT).

METHODS

BALB-neuT female mice spontaneously develop mammary tumors. Starting at weaning, Cd was administered in drinking water. Tumor-free survival, tumor multiplicity, and survival were then compared in Cd-treated and control mice. The absence of acute Cd toxicity was evaluated. Additionally, histological and immunohistochemical (IHC) analyses of mice mammary tissues were performed. IHC analysis assessed the expression of markers including progesterone receptor (PR), ER, GRP30 receptor, and CD31, and of proteins involved in cell proliferation signaling such as Neu, AKT, and Ki67, and of markers of immune infiltrating cells.

RESULTS

Cd increased the number of tumors per mouse and reduced both disease-free survival and overall animal survival. Cd significantly increased the expression of PR and Ki67. Analysis of Neu and GPR30 showed the overexpression of both markers without difference in all samples analyzed, while αER was negative in all samples. Studies of the tumor-infiltrating immune cells ($CD4^+$, $CD8^+$) showed an increasing trend in samples collected from Cd-treated mice.

CONCLUSIONS

Oral daily intake of Cd significantly accelerated neoplastic transformation in BALB-neuT transgenic mice, enhanced cell proliferation and affected infiltrating tumor cells in the BALB-neuT mouse model. These findings highlight the role of Cd as an endocrine disruptor in promoting breast cancer development and progression, emphasizing the need for stringent regulation of environmental cadmium exposure.

P108

EPICARDIAL ADIPOSE TISSUE IN TYPE 2 DIABETES: A MODIFIABLE RISK FACTOR FOR CARDIOVASCULAR COMPLICATIONS

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BACKGROUND-AIM

Many type 2 diabetic (T2D) patients have a high incidence of major risk factors, such as cardiovascular diseases, which emphasizes the urgent need to develop novel approaches to prevent these complications. Epicardial adipose tissue (EAT) is abnormally increased in patients with T2D with harmful effects on the adjacent myocardium via secretion of pro-inflammatory and pro-fibrotic cytokines, following cardiac remodeling and heart failure. Despite this, the predictive potential of EAT in general, and in patients with diabetes, is yet to be established, and the clinical relevance of EAT is therefore limited.
This study aims to pose the molecular evidence to translate the EAT into the clinical scenario as modifiable risk factor to be targeted with pharmacological approaches.

METHODS

Blood samples and EAT biopsies were collected from non-diabetic (ND) coronary artery disease (CAD) patients (n=37) and T2D CAD patients (n=20) enrolled during bypass surgery. Cytokines and chemokines secretion were measured using a multiplex assay.
From a subgroup of patients, EAT-derived mesenchymal stem cells (MSCs) were isolated, cultured, and differentiated. mRNA expression of specific genes was measured by real-time RT-PCR. Moreover, senescence-associated beta-galactosidase (SA-βgal) activity has been evaluated by a fluorescence-based assay using flow cytometry.

RESULTS

IL-1β, IFN-γ, IP10, and Eotaxin were increased in serum from T2D CAD patients. In parallel, EAT biopsies from diabetic CAD patients secrete higher levels of pro-inflammatory cytokines, such as IL-6, IL-8, IL-1β, TNF-α, and MCP-1, compared to ND.
In T2D, EAT-derived MSCs exhibited impaired differentiation towards adipogenic and osteogenic lineages. This phenotype is accompanied by multiple senescence markers, like gain of CDKN1A and loss of LMNB1 expression and increase in the percentage of SA-βgal positive cells.
The treatment with senolytic combination of Dasatinib plus Quercetin on T2D-derived cells reduced senescence-associated secretory phenotype in the media compared to the media conditioned by vehicle.

CONCLUSIONS

These results provided significant evidence linking local inflammation, cellular dysfunction and senescence to worsening atherosclerotic coronary artery disease in patients with T2D.

P109

EVALUATING THE ROLE OF TERRA LEVELS IN PERIPHERAL BLOOD MONONUCLEAR CELLS AS A PROGNOSTIC BIOMARKER IN ELDERLY COLORECTAL CANCER PATIENTS

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BACKGROUND-AIM

Aging is associated with several diseases, including cancer. Evaluating aging biomarkers in peripheral blood is essential for minimally-invasive monitoring of frail patients. Senescent cells exhibit reduced replicative capacity, primarily due to telomere shortening or dysfunction. TERRA, a class of long noncoding RNA transcribed from telomeres, sustains various functions at telomeres and may impact cellular senescence. Here, we investigated the role of TERRA in peripheral blood mononuclear cells (PBMC) as a potential biomarker for senescence and prognosis in 70 elderly colorectal cancer (CRC) patients (aged ≥ 70 years) at the time of surgery (baseline) and in 50 of them also one year after tumor resection (follow-up).

METHODS

TERRA transcripts originating from chromosomes 1q-2q-10q-13q (TERRAch1-2-10-13), 15q (TERRAch15), 20q (TERRAch20), and XpYp (TERRAchXY) were quantified by RT-PCR and analyzed in relation to other biomarkers, including T-cell immunophenotype, thymic output, telomere length, denervation biomarkers and senescence-associated secretory phenotype (SASP), as well as clinical outcome.

RESULTS

At baseline, TERRA levels tended to correlate with telomere length (TERRAch1-2-10-13, rs=0.279, p=0.081; TERRAch15, rs=0.298, p=0.061; TERRAch20 rs=0.257, p=0.109; TERRAchXY rs=0.366, p=0.02). Notably, patients with higher TERRAch15 levels (above the median value) had significantly lower risk of adverse event (relapse, progression, or death) (hazard ratio: 0.369 p=0.028) and showed significantly lower levels of CD8 T cells, including senescent (CD8+CD28-CD57+, p=0.014), naïve (CD8+CD45RA+, p=0.035), recent thymic emigrants (CD8+CD45RA+CD31+, p=0.026) cells, and higher CD4/CD8 ratio (p=0.038) compared to patients with lower TERRAch15 expression. At follow-up, TERRA levels inversely correlated with SASP markers. Specifically, TERRAch1-2-10-13 and TERRAch20 were inversely correlated with IL8 (rs=-0.407, p=0.048 and rs=-0.447, p=0.042, respectively), and TERRAch20 and TERRAchXY were inversely correlated with CXCL-1 (rs=-0.467, p=0.033 and rs=-0.608, p=0.002, respectively).

CONCLUSIONS

These findings suggest that TERRA levels in PBMC could be useful for monitoring “biological aging” and represent a promising prognostic marker for elderly CRC patients.

P110

CREATION OF A GASTRIC CANCER ORGANOID CULTURE COLLECTION FOR THE STUDY OF CHEMORESISTANCE AND TARGETED THERAPY

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BACKGROUND-AIM

Gastric cancer (GC), originating from the epithelial cells of the gastric mucosa, is the fifth most common cancer and the fourth leading cause of cancer-related deaths globally. Traditional two-dimensional cell cultures of stomach cancer have very low yield, and the few available cultures are often derived from Asian patients, metastases, or passaged through murine models, limiting their representation of primary tumors and introducing bias. The breakthrough by Sato et al. 15 years ago in long-term organoid cultures from adult intestinal stem cells, combined with recent advances in 3D culture techniques, has led to more effective methods for studying gastric cancer.

METHODS

Biopsies were collected from patients undergoing surgery at AOU Careggi who had given consent to the relevant ethics committee. For each patient, both healthy and tumor mucosa were collected and processed to obtain organoids and preparations for immunohistochemistry.
Slight differences in processing healthy and tumor mucosa occur. For tumor tissue, an enzymatic matrix digestion using collagenase and hyaluronidase is performed, while for healthy tissue, a chelation buffer that softens the matrix and pressure to release gastric glands into the medium have been used.

RESULTS

It was possible to establish organoid cultures from 100% of the healthy tissue, while cultures were obtained from about 50% of the tumor samples. Immunohistochemical analyses of the primary biopsy and the obtained organoids confirmed a correspondence in morphology and markers between the samples from the same patient.

CONCLUSIONS

Although the literature reports an efficiency of about 80% for the establishment of gastric tumor organoids, these numbers refer to samples obtained from patients who had not received any treatment. In our case, most of the patients had already undergone perioperative chemotherapy, which might explain the lower yield. Additionally, the tumor cultures were subjected to selection by depleting certain factors from the culture medium that are essential for organoids from healthy tissue but which tumor cells may have become independent of during the carcinogenesis process. This further reduced the number of cultures obtained. Nevertheless, the establishment of gastric tumor organoid cultures from patients, even those who had undergone chemotherapy before the explant, will allow the study of chemotherapeutic response in vitro and its correlation with the patient’s response in vivo.

P111

ADIPOCYTE-RELEASED FABP4 ENHANCES RENAL CANCER CELL MOTILITY

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BACKGROUND-AIM

Clear cell Renal Cell Carcinoma (ccRCC) is the most common and lethal subtype among renal cancers. Several epidemiological studies report a strong association between excessive body weight and both ccRCC incidence and prognosis. These epidemiological data suggest that Peri-Renal Adipose Tissue (PRAT) may affect ccRCC behavior through the exchange of proteins, cytokines and metabolites with cancer cells. In the present study we investigated the effect of adipocyte-released FABP4 on ccRCC.

METHODS

We collected Adipocytes Conditioned media (AD-CM) using PRAT from 19 ccRCC patients and 10 Healthy Donors (HD) undergoing radical nephrectomy. We evaluated migration in 786-O and ACHN incubating cells with recombinant FABP4, Ad-CM from ccRCC and Ad-CM from HD alone or in combination with FABP4 inhibitor BMS309403. Cell migration was evaluated using scratch assays. In addition, we evaluated pERK and ERK expression with Western Blot.

RESULTS

Our results showed that Ad-CM from ccRCC significantly increased cell motility in both ACHN and 786-O cells (p 0.0006 and 0.007, respectively), this effect was partially reverted by FABP4 inhibitor BMS309403 in both cell lines (p 0.0173 and p 0.0178 respectively). Finally, in 786-O cell line, FABP4 effect was associated with an increased ERK phosphorylation (p 0.0224).

CONCLUSIONS

Cancer cell migration is a key step in the metastatic spread. To the best of our knowledge, our study showed for the first time that FABP4 released by peri-tumoral adipocytes promote renal cancer cell migration, suggesting that it may represent a therapeutic target in ccRCC patients.

P112

IMPACT OF CHRONOTYPE ON INFLAMMATORY MARKERS AND CLOCK GENE EXPRESSION IN ADIPOSE TISSUE OF SEVERELY OBESE SUBJECTS

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BACKGROUND-AIM

Individual circadian preferences in behavioural and biological rhythms are commonly referred to as chronotype. The morning chronotype (MC) tends to wake up early and prefers activities at the beginning of the day, while the evening chronotype (EC) generally wakes up later and prefers its main activity in the late afternoon/evening. The intermediate chronotype (IC) occupies an intermediate position. Subjects with EC are more prone to develop metabolic diseases compared to subjects with MC. The aim of this study was to investigate if chronotype may impact on adipose tissue (AT) circadian clock and secretory pattern, contributing to obesity-related comorbidities.

METHODS

25 severely obese patients undergoing bariatric surgery were enrolled and subjected to anagraphical, clinical, and lifestyle phenotyping. The Morningness-Eveningness Questionnaire (MEQ) was used to assess the patients' chronotype (EC MEQ:<41; IC MEQ:42-58; MC MEQ>59). Serum samples, as well as subcutaneous (SAT) and visceral (VAT) AT biopsies, were obtained at the time of surgery. Serum and SAT- and VAT-conditioned media (CM) were screened for an array of 27 cytokines, chemokines, and growth factors, based on a multiplex approach. CLOCK, PER1, and BMAL1 gene expression were measured by qPCR on SAT and VAT biopsies.

RESULTS

The population was stratified according to chronotype (EC N=7; IC N=9; MC N=9). MC displayed an increase in Neutrophiles to Lymphocytes Ratio and slightly higher levels of circulating IL-1β, IL-2, IL-4, IL-9, IL-15, IL-17, bFGF, GM-CSF, IFNy, and TNFα ($p<0.05$). No correlation between clock genes expression and MEQ score was detected in SAT and VAT. Interestingly, in VAT-CM, IL-1β, IL-4, IL-6, IL-9, bFGF, MCP-1, MIP-1β, RANTES, and TNFα resulted higher in EC with respect to MC ($p<0.05$), with a significant negative correlation with MEQ score. In addition, VAT-CM, compared to SAT-CM, showed an increase of IL-1β, IL-4, IL-17, and GM-CSF in EC ($p<0.05$), with no differences in MC and IC.

CONCLUSIONS

In severely obese subjects, although MC displays a more imbalanced systemic inflammatory profile, EC impacts largely on VAT features, leading to an increase of local inflammatory mediators' release which may contribute to the development of obesity-associated metabolic comorbidities.

P113

COMBINED LOW-DOSE TREATMENT WITH IFN-γ/TNF-α RENDERS BREAST CANCER CELLS MORE SUSCEPTIBLE TO NATURAL KILLER CELL-MEDIATED KILLING.

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BACKGROUND-AIM

Identifying the molecular mechanisms that make tumor cells more susceptible to immune surveillance is a significant challenge for oncoimmunologists to optimise current immunotherapies. We explored novel immunomodulation mechanisms to induce and promote more effective recognition and elimination of tumor cells by Natural Killer (NK) cells. Specifically, we assessed the effect of the combination of two cytokines, IFN-γ and TNF-α, used at low pre-apoptotic dose, on the surface expression of ligands recognized by NK cell activating receptors, as well as Fas and TRAIL-R2 death receptors and ICAM-1 adhesion molecule in MDA-MD-468, MDA-MD-231, and MCF-7 breast cancer (BC) cell lines. Furthermore, we evaluated the impact of IFN-γ and TNF-α treatment on the susceptibility of BC cell lines to NK cell-mediated responses.

METHODS

The experimental strategy involved in vitro cytokine treatment of BC cell lines to: i) analyze the expression of ligands for NK cell activating receptors, apoptotic death receptors ICAM-1, as well as the phenotype and functions of NK cell infiltrating BC spheroids in response to IFN-γ and TNF-α through flow cytometry; ii) investigate the NK cell-mediated anti-tumor response through functional assays of apoptosis, degranulation, and conjugate formation; iii) evaluate NK cell infiltration into BC spheroids by microscopic acquisition and analysis.

RESULTS

Combined low-dose IFN-γ and TNF-α treatment of BC cell lines resulted in i) increased expression of apoptotic death receptors Fas and TRAIL-R2, which led to increased NK cell-mediated apoptosis of BC cells; ii) a substantial increase in ICAM-1, which induced increased conjugate formation between BC cells and NK cells; iii) increased NK cell ability to infiltrate spheroids; iv) increased NK cell-mediated apoptotic state and reduced size of spheroids; iv) increased expression of activating receptors and reduced PD-1 in spheroid-infiltrating NK cells.

CONCLUSIONS

These results highlight how the combined low-dose treatment of IFN-γ and TNF-α can positively influence NK cell infiltration into the tumor microenvironment (TME), thus providing a rational for further investigations to design new immunotherapeutic strategies based on NK cells and immunomodulating cytokines for the treatment of BC.

P114

SARS-COV-2 ENVELOPE PROTEIN ACTS AS MEMBRANE-ASSOCIATED ION CHANNEL PROTEIN AND INTRACELLULAR MODULATOR OF CALCIUM CONTENT: RELEVANCE FOR HUMAN CELL CYCLE PROGRESSION AND PLURIPOTENCY

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BACKGROUND-AIM

COVID-19 is a pathological condition causing damages not only to the respiratory system but also to other organs, whose functions are adversely compromised. Indeed, recent studies have highlighted the ability of SARS-CoV-2 virus to infect numerous cellular phenotypes beyond pneumocytes, such as cardiomyocytes, neurons, and stem cells. In particular, structural proteins of SARS-CoV-2 such as the Envelope (E) viroporin have been identified in infected cells. E viroporin is repoted to act as non-selective channel protein that is necessary for virus biology and hypothesized to serve as primary cause of functional derangements of host cells.

METHODS

We have first generated lentiviral vectors for the overexpression of SARS CoV 2 E protein and validated the vector on HEK293 cells. In addition, we obtained human induced pluripotent stem cells (hiPS-Cs) stably expressing the construct. On these cellular models we have studied the E protein localization, performed Ca2+ imaging experiments and characterized the viability, proliferation rate and assessed the pluripotency.

RESULTS

Using lentiviral vectors we obtained HEK293 cells and human induced pluripotent stem (hiPS) cells stably expressing the SARS-CoV-2 E viroporin. Immunofluorescence of HEK and hiPS cells revealed E viroporin localizes into the plasmalemma as well as in the intracellular compartment, showing a distribution associated to the endoplasmic/sarcoplasmic reticulum. Accordingly, patch-clamp recordings showed E viroporin mediates an outward potassium current associated to the plasmalemma, while calcium imaging assay uncovered a depletion effect of E viroporin on intracellular stores. These effects are associated to a consistent reduction of cell viability, proliferation and a clear-cut slowing of cell cycle progression with a notable increase of cells in the G1/G0 phase. These modifications also affect the expression of differentiation markers during hiPS cells mesodermal differentiation.

CONCLUSIONS

Our results demonstrate E viroporin acts as a membrane-associated ion channel protein and endoplasmic/sarcoplasmic modulator of cell calcium content. These roles are associated and likely sustain the reduction of cell cycle progression. Pluripotency resulted partially reduced, suggesting an implication of SARS-CoV-2 E in tissue regeneration effiency.

P115

INVESTIGATING THE REGENERATION COMPETENCE OF PERIPHERAL MOTOR NEURONS IN ALS

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BACKGROUND-AIM

Amyotrophic lateral sclerosis (ALS) is spectrum of diseases with adult onset, multiple etiologies, wide phenotypical heterogeneity, and inadequate understanding of pathological mechanisms, which hampered the development of efficacious therapeutical interventions. The neuromuscular junction (NMJ) is the first target of the pathology in both patients and animals modelling the disease. Distal denervation begins very early, prior to the onset of symptoms, and precedes motor neuron death in the spinal cord. Motor unit loss is preceded by a plastic remodelling of the NMJs, which undergo cycles of denervation/re-innervation, until regenerative processes are progressively overwhelmed by degenerative events. While motor axon terminal degeneration in ALS has been widely studied, very little is known about the regenerative capability of the system that we addressed in the present study. The ability of ALS NMJs to remodel for some time suggests that supporting/prolonging synaptic plasticity and regeneration can be therapeutically exploitable to delay denervation.

METHODS

We assessed the regeneration competence of mutant motor neurons by exposing SOD1G93A mice at different disease stages to the acute but reversible degeneration of their motor axon terminals caused by the presynaptic neurotoxin α-Latrotoxin (α-LTx). Recovery of neuromuscular function was followed over time by electrophysiology and imaging.

RESULTS

We found that the regenerative capability of SOD1G93A NMJs is almost fully preserved at an early symptomatic stage and then progressively declines as symptoms worsen.

CONCLUSIONS

We thus identified a 'regeneration competence time window' within which to stimulate the regenerative capability of the system, in order to stabilize the NMJs and delay denervation, and, in turn, counteract disease progression.

P116

CYTOKINES FROM SPIKE-ACTIVATED MACROPHAGES HINDER PROLIFERATION AND CAUSE CELL DYSFUNCTION IN ENDOTHELIAL CELLS

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BACKGROUND-AIM

Endothelial dysfunction plays a central role in the severity of COVID-19, since the respiratory, thrombotic and myocardial complications of the disease are closely linked to vascular endothelial damage. In this context, growing evidences sustain a pivotal role for the hyper-secretion of infiammatory molecules (the socalled “cytokine storm”) in the onset of endothelial damage. To address this issue, we explored the effect of a cytokine-enriched conditioned medium from Spike S1-activated macrophages (CM_S1) on the proliferation and viability of human umbilical endothelial cells (HUVECs), focusing on the specific role of IL-1ß, IL-6, IFNγ and TNFα.

METHODS

Human macrophages, derived from circulating monocytes, were left untreated or incubated with Spike S1 from SARS-CoV-2 for 24 h to obtain conditioned media (CM and CM_S1, respectively). HUVECs were then exposed to CM_S1 or to 5 ng/ml of IFNγ or IL-1ß, or 50 ng/ml of TNFα or IL-6, alone or combined. Cell proliferation was assessed by counting the number of cells, while cell cycle and death were determined through flow cytometer through staining with propidium iodide and Annexin V FITC/Dead Cell Apoptosis Kit. The expression of NF-κB p65, IκBα, STAT1, IRF1 and p21WAF was assessed with Western Blot, while the mRNA level of BAK1, BCL2, BID, CDKN1A, CDKN1B and IRF1 were evaluated with RT-qPCR.

RESULTS

The incubation of HUVECs with CM_S1 for 72 h hindered endothelial cell proliferation and induced signs of cell death. When testing the effects of cytokines, comparable results have been obtained only upon exposure to a combination of IFNγ and TNFα. These events associate with an increase in p21 protein and a decrease in Rb phosphorylation, as well as with the activation of IRF-1 and NF-kB transcription factors.

CONCLUSIONS

These findings i) demonstrate that Spike-activated macrophages exerts anti-proliferative/pro-apoptotic effects in HUVECs, likely through the synergism of IFNγ and TNFα, and ii) sustain a role for these cytokines in the immune-mediated endothelial dysfunction in COVID-19.

P117

INVESTIGATING EPHA2 DYNAMICS IN CHEMOTHERAPY-TREATED COLORECTAL PATIENT-DERIVED ORGANOIDS

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BACKGROUND-AIM

EphA2 is highly expressed in colorectal cancer (CRC), and CRC patients with high EphA2 expression have a worse prognosis. Patient Derived Tumor Organoids (PDTO) are 3D structures known to mimic closely the heterogeneous cyto-architecture of the originating specimens Here we have investigated the contribution of EphA2 to organoid dynamics at steady state and under oxaliplatin treatment.

METHODS

PDTO cultures setting and propagation, flow cytometry and microscopy analysis of EphA2pos cell subpopulations and live/dead cell staining. ALDH activity and clonogenic assays. Western blotting and Indirect ELISA assays.

RESULTS

EphA2-positive cells represented a stable cell subpopulation in CRC PDOs that persisted over time and through passaging. Given the involvement of EphA2 in mediating response to chemotherapy, we challenged the PDOs with oxaliplatin (OXA) at pharmacologically relevant schedules. This revealed the persistence of EphA2-positive cells in both acutely and chronically (21dd) treated cells. To investigate this observation, we analyzed EphA2 protein levels and its phosphorylation status using zn-phos tag gels and indirect ELISA assays. This revealed that ser897 of EphA2 was an oxaliplatin-sensitive phosphorylation modification in CRC PDOs, which correlated with increased EphA2 protein levels. Functionally, we found that RNAi-mediated knockdown of EphA2 or attenuated EphA2 ser897 phosphorylation significantly reduced the organoid forming ability (OFA) of PDOs after OXA treatment, consistent with a chemosensitizing effect. Specifically, we identified RSK1 and MK2 as the kinases responsible for OXA-induced EphA2 phosphorylation at Ser897. We also present preliminary data obtained in HCT116 CRC cells to mechanistically address the observed phenomenon, with a focus on EphA2 levels and their relationships with the protumorigenic, chemotherapy-induced, senescence-associated secretory phenotype (SASP).

CONCLUSIONS

We speculate that EphA2 contributes to the adaptive response of CRC PDOs to OXA and that phosphorylation of EphA2 ser897 may be instrumental in such modulation.

P118

EXPLORING NOTCH3 EXPRESSION IN DIFFERENT GRADE HEPATOCELLULAR CARCINOMAS AND ITS INTERPLAY WITH KDM2A

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BACKGROUND-AIM

Notch3 receptor is involved in different aspects of liver carcinogenesis. Nevertheless, to unlock Notch3 therapeutic/diagnostic/prognostic potential a deeper understanding of its role in hepatocellular carcinoma (HCC) onset and progression is needed. KDM2A (lysine 36 histone H3 demethylase) plays a key role in epigenetic chromatin remodeling and gene transcription. Its levels increase together with HCC grading. We investigated the involvement of KDM2A in controlling Notch3 expression in HCC.

METHODS

An expression analysis of Notch3 and KDM2A was conducted by Real time PCR on mRNA from formalin-fixed paraffin-embedded (FFPE) HCCs and peritumoral tissue (PT). A human HCC cell-line, Huh7, expressing Notch3 and KDM2A, was transiently silenced for KDM2A using a specific siRNA to perform a first evaluation of Notch3/KDM2A association. The levels of KDM2A and Notch3 after the silencing were assessed by Real-Time PCR and Western-Blotting (WB). Immunohistochemistry (IHC) on HCCs was conducted using anti-Notch3 and anti-KDM2A antibodies.

RESULTS

Notch3 and KDM2A levels assessed on FFPE samples revealed an increased expression in HCCs compared to PT ($p<0.001$ and $p<0.01$ respectively) and going from G1 to G3 HCC. IHC showed a diverse localization of Notch3 based on the differentiation state. In well differentiated HCC the staining was mainly localized in the vascular endothelium while in poorly differentiated HCC it involved clusters of tumoral hepatocytes, sometimes invading portal areas. The staining with CD34 showed that the Notch3 positive blood vessels were consequences of neo-angiogenesis. The transient KDM2A silencing performed on Huh7 interestingly resulted in a downregulation of Notch3 transcript ($p\leq0.001$), confirmed by WB ($p\leq0.01$).

CONCLUSIONS

These findings showed an overall increasing trend of Notch3 expression during HCC progression. IHC revealed an involvement of Notch3 in neo-angiogenesis even in early steps of liver carcinogenesis and a role in local invasiveness of stromal portal areas in G3 tumors. Furthermore, we found an association between Notch3 and KDM2A suggesting a new possible mechanism of epigenetic regulation that could be responsible for the higher Notch3 expression in poorly differentiated HCC with high levels of KDM2A.

P119

NEW PRECURSORS OF THE HIV PROTEASE INHIBITORS EXERT ANTI-TUMOR ACTIVITY AGAINST HEMATOLOGIC CANCERS BY MODULATING MITOCHONDRIAL ACTIVITY AND AUTOPHAGY

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BACKGROUND-AIM

Myeloma and chronic lymphocytic leukemia (CLL) are two common hematological cancers that often have poor prognosis due to drug resistance. Therefore, there is an urgent need to develop new drug therapies for these patients. Drug-resistant myeloma and CLL cells have increased mitochondrial metabolism and autophagic flux, making them potential targets for reversing drug resistance. HIV-1 protease inhibitors have antitumor properties and beneficial effects when combined with other therapies. In this study, we developed and tested two new Darunavir precursors, RDD-19 and RDD-142, for their antitumor properties in myeloma and CLL tumor cell lines and characterized their mechanisms of action.

METHODS

Two multiple myeloma cell lines (MM1S, RPMI-8226) and a CLL cell line (HG3) were treated with RDD-19 and RDD-142 at 30 μM for 48h. Peripheral blood mononuclear cells (PBMCs), isolated from healthy donators were used as controls. Using flow cytometry, cell cycle analysis was performed with propidium iodide (PI), and the apoptosis assay was performed using Annexin V-FITC/PI staining. Moreover MM1S, RPMI-8226, HG3, and PBMCs were treated with RDD-19 and RDD-142 at 30 μM for 24h to measure in real time the oxygen consumption rate (OCR) and pH extracellular acidification rate (ECAR), respectively mitochondrial respiration and glycolysis, by Seahorse ATP rate assay kit (Agilent Seahorse XF-Pro Analyzer).

RESULTS

RD19 and RD142 exhibited selective cytotoxicity towards myeloma and CLL cancer cells relative to normal peripheral blood cells derived from healthy individuals. These drugs induce cell cycle arrest at the G1 phase and trigger apoptosis. The cytotoxicity of these two drugs is mediated by the inhibition of oxidative phosphorylation and autophagy, which are highly active in these tumor lines and are linked to drug resistance. Furthermore, these findings were replicated in myeloma cells derived from patients who were sensitive or resistant to proteasome inhibitors. Given their reduced toxicity in PBMCs, both drugs may have fewer side effects and could be considered for clinical use in the treatment of myeloma and CLL.

CONCLUSIONS

RDD-19 and RDD-142 exert their anti-tumor effects by impairing mitochondrial respiration and autophagy in myeloma and CLL cells, ultimately leading to G1 cell cycle arrest and apoptosis. Compared to PBMCs, myeloma and CLL cells are more sensitive to these drugs, making them promising novel therapeutic options for the treatment and potential cure of malignancies.

P120

REPURPOSED DRUG CANDIDATES MEBENDAZOLE AND NITROXOLINE IN COMBINATION EXERT ANTIPROLIFERATIVE EFFECTS IN PANCREATIC CANCER CELLS

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BACKGROUND-AIM

Pancreatic cancer (PC) is one of the most leading causes of cancer-related death in the world and represents a major challenge in cancer patient management, due to a very limited response to the currently available anticancer therapies. Thus, more effective therapeutic options are needed to improve PC patient outcome. In this scenario, drug repurposing is an attractive strategy to expand the repertoire of agents which might be eventually exploited in the clinic. Mebendazole (MBZ) and nitroxoline (NTX) are promising non-anticancer drugs that showed to be effective as single agents in several preclinical cancer models, including PC. Here, we evaluated the antiproliferative effects of MBZ and NTX in combination using distinct PC cell lines.

METHODS

Antiproliferative effects of MBZ and NTX in combination were evaluated in five PC cell lines (AsPC-1, BxPC-3, Capan-1, L3.6Pl, PATU8902) with distinct genetic profiles. Viability and self-renewal capacity of PC cells after treatments were assessed by MTT and clonogenic assays, respectively. Additional analyses to unravel the effects of MBZ/NTX combined treatments on PC cells are ongoing.

RESULTS

Our preliminary results showed that combinations of MBZ/NTX inhibited PC cell viability and clonogenicity in a dose-dependent manner, although with distinct sensitivities across the five PC cell lines. Notably, some of the combined treatments induced a significant reduction of cell viability and/or clonogenicity, compared to the effects of single agents at the corresponding concentrations.

CONCLUSIONS

These preliminary results provide clues on the potential value of mebendazole/nitroxoline combined treatments in the search for effective agents in PC treatment.

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P121

ANTI-CN1A IN THE REAL LIFE: IS IT TRULY A MYOSITIS SPECIFIC ANTIBODY?

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BACKGROUND-AIM

Anti-cytosolic 5'nucleotidase 1A (anti-cN1A) autoandibodies were proposed as specific diagnostic markers for inclusion body myositis (IBM), but they were also demonstrated in other autoimmune diseases and to a small extent in healthy people. Our study aims to verify the diagnostic accuracy of anti-cN1A, analyzing its prevalence and clinical correlation in patients who came to our attention for suspected idiopathic inflammatory myopathies (IIM).

METHODS

We recently introduced in our laboratory a test to assess the presence of anti-cN1A antibodies by lineblot method as part of an extended profile (Euroline 9G by Euroimmun) for autoantibodies associated with autoimmune myopathies. We collected laboratory and clinical features of all cN1A positive cases from nov 2022 to apr 2024.

RESULTS

40 patients (82.5% females; mean age=50.5±25.0 yrs, range 3-86 yrs) tested positive for anti-cN1A. 20 were high positive, 12 moderate and 8 low. Only 18/40 (45%) were positive only for cN1A, while the majority presented also other autoantibodies (mostly Ro60 and Ro52, but also PM/Scl75 and 100, U1RNP, Scl70, Ku, Jo1, SSB, HA, MJ/NXP2 and Mi2-beta). Definite clinical data were available in 36 cases. Only 3/36 (8.3%) patients were diagnosed as myopathies (one polymyositis, one in overlap with scleroderma and MCTD, one antisynthetase sdr in overlap with Sjogren's syndrome), while 13 presented other connective tissue diseases (lupus, scleroderma, vasculitis, polymyalgia, fibromyalgia), 15 other non-rheumatic diseases and 5 nothing relevant. Anti-cN1A antibodies were high positive both in myopathies and in unrelated diseases.

CONCLUSIONS

In our experience, anti-c1NA are rarely present in isolation and as myositis-specific antibodies. They can be frequently found at high titer also in other autoimmune and non autoimmune diseases.

P122

HOPS/TMUB1 IN MITOTIC REGULATION: INSIGHTS INTO ITS ROLE IN CELL DIVISION AND ONCOGENESIS

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BACKGROUND-AIM

The cell cycle encompasses a series of orchestrated events essential for the accurate division of eukaryotic cells. The progression into each phase is meticulously regulated by checkpoints that can halt the cycle to enable correction mechanisms or, if the correction fails, lead the cell to apoptosis or senescence. When a cell circumvents these regulatory systems, it gains the capability to replicate uncontrollably and grow abnormally, a hallmark of oncogenesis. Central to this context is the ubiquitin-like modifier HOPS/TMUB1 (hepatocyte odd protein shuttle/transmembrane and ubiquitin-like domain-containing protein 1), which plays a pivotal role in centrosome assembly. This study highlights the regulatory role of HOPS during mitosis.

METHODS

$Hops^{+/+}$ and $Hops^{-/-}$ mouse embryonic fibroblasts (MEFs) were derived from C57BL/6 and $Hops^{-/-}$ C57BL/6 mice, respectively. $Hops^{-/-}$ HeLa cells were generated via CRISPR/Cas9. HOPS targets were identified and validated using LC-MS, co-immunoprecipitation and immunofluorescence assays.

RESULTS

The interactome of HOPS with mitotic regulatory proteins such as RhoA, Anillin, RacGAP1 and KIF23 was revealed for the first time during mitosis in HeLa cells. $Hops^{-/-}$ cells show a delay in cell growth compared to the control ($Hops^{+/+}$). In particular, HOPS is highly concentrated at the intercellular bridge during cytokinesis, especially in the midbody. Analysis of midbody lysates from $Hops^{-/-}$ HeLa cells revealed reduced expression of HOPS interactors, resulting in impaired stability and delayed cell division. The impact of HOPS on cytokinesis is demonstrated by in vitro and in vivo knock-out models. A distinct HOPS-dependent phenotypic alteration in indicators of mitotic failure, such as enlarged nuclei and increased binucleation, is observed. Furthermore, the liver of $Hops^{-/-}$ mice showed multiple aberrant mitoses characterized by a disorganized cytoskeleton, multipolar spindles, dispersed chromatin and abnormal chromosome segregation.

CONCLUSIONS

HOPS emerges as a critical factor in ensuring and promoting proper cell division. Its involvement in vital regulatory steps positions it as a potential target in the intricate process of cancer progression, providing new insights for therapeutic intervention in oncology.

P123

APPLICATION OF BIOLOGICAL AGE ESTIMATION IN DIFFERENT CLINICAL SETTINGS: RESULTS AND FUTURE PERSPECTIVES OF A WELL-ESTABLISHED CLASS OF PROMISING BIOMARKERS

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BACKGROUND-AIM

Aging is an established and shared risk factor among the most common diseases. Recently biological age estimation has proven to be able to integrate chronological age in describing a person's actual health status. In our body different molecular clocks exist that sign the pace of individual biological aging that is intimately linked with the age-related fitness impairing, that in turn pave the way to the onset of most of the diseases of adulthood and elderly. Here we present published and unpublished results from the application of biological age estimation methods in different clinical settings.

METHODS

We estimated the biological age using epigenetic clocks, i.e. algorithms that combine DNA methylation levels of sets of CpG sites into an individual biological age estimation. DNA methylation levels were assessed by Illumina genome-wide arrays and targeted approaches. Epigenetic Age Acceleration (EAA) is calculated as the difference between predicted age and chronological age and positive and negative EAA values indicate a faster or slower aging process, respectively.

RESULTS

We applied DNA methylation clocks to the following cohorts:
1. extreme longevity, i.e. semi-supercentenarians (EAA CENT vs CTR: p=0.029) and their offspring (EAA OFF vs CTR: p=0.016),
2. effect of diet (EAA before vs after nutritional intervention: p=0.031),
3. neuropatic pain in Type 2 Diabetes (EAA painful vs painless: p=0.005),
4. HIV patients under C-ART treatment (EAA persons living with HIV vs CTR: p=0.004),
5. Parkinson disease patients (EAA advanced PD vs CTR: p=0.013),
6. Alzheimer's disease patients (EAA AD vs CTR: p=NS),
7. Down Syndrome subjects (EAA DS vs CTR: p=1.2*10−3),
8. Isolated REM Sleep Behavior Disorder patients (EAA iRBDs vs CTR: p=0.006),
9. B-cell lymphoma patients infused with CD19.CAR-T cells (EAA before CAR-T infusion vs CAR-T expansion peak: p=0.011),
10. recipients of allogenic Hematopoietic Stem Cell Transplantation (EAA before HSCT vs 1 year after HSCT: p=0.0004).

CONCLUSIONS

Our results show that the pace of aging is accelerated in the pathological conditions that we analyzed, in agreement with a huge body of literature. Biological age also appears to be efficient in capturing healthy aging phenotypes and interventions capable to improve the quality of aging process.

P124

INSL4 EXPRESSION REVEALS AN ONCOEXAPTED TRANSCRIPTIONAL PROGRAM IN LUAD TARGETABLE BY EPIGENETIC MOLECULES

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BACKGROUND-AIM

LUAD is the leading cause of cancer related death in both genders. Previous studies allowed us to classify INSL4, a placenta specific gene, as an oncogene. INSL4 is overexpressed in several LUAD cell lines (e.g. A549) and in nearly 20% of LUAD patients in which correlates with worst OS. Here, we investigate the epigenetic control of INSL4 in LUAD in order to target it pharmacologically.

METHODS

Bioinformatic analyses were performed using UCSC Genome Browser, CCLE and IGV-Tool. Gene Expression experiments were carried by qPCR and RNA-Seq. Drug Screening were performed through CFA.

RESULTS

A bioinformatic analysis of the INSL4 highlighted, upstream of the gene, the presence of LTR22B1, a Transposable Element (TE), with probable enhancer activity on it, a phenomenon known as Oncoexaptation. TEs are epigenetically silenced via DNA methylation, so we proceeded by analyzing the methylome of 53 LUAD cell lines. This analysis showed that overexpression of INSL4 was highly correlated with hypomethylation of LTR22B1. Through the IGV-Tool, we thoroughly characterized the epigenetic profile of LTR22B1 in A549 by phenotyping it as an enhancer. Based on this evidence we treated A549 with iBET and iSMARCA2/4 at 1 μM. These molecules were then combined (Combo). Through qPCR we observed the ability of these molecules to reduce the expression of INSL4 individually in an extremely significant manner. The effect was even greater with Combo, which is able to completely switch off the gene. A549 were treated with Combo to obtain a complete Gene Expression profile by means of RNA-Seq. We observed significant downregulation of 2853 genes. Through intersection analysis –using the same epigenetic profile as LTR22B1– we discovered an additional 585 potentially oncoexapted genes downregulated by Combo. After 15 days with only 3 treatments Combo completely eliminates A549 in CFA as well as in the INSL4 overexpressing cell lines: H1944 and H1568.

CONCLUSIONS

These findings suggest the existence of a large Oncoexapted Transcriptional Program that could play a fundamental role in LUAD. The combined use of iBETs and iSMARCA2/4 is extremely effective in shutting down this program and completely eliminating A549, representing a promising therapeutic approach for INSL4 overexpressing LUADs.

P125

ACTIVATED HUMAN MACROPHAGES ARE POTENTIAL GLUTAMINE SUPPLIERS FOR CD8+ T-CELLS

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BACKGROUND-AIM

L-Glutamine (Gln) plays a pivotal role in the activation of macrophages and lymphocytes. At variance with macrophages, T lymphocytes do not synthetize Gln through Glutamine Synthetase (GS), suggesting that they depend on transport of the extracellular amino acid. However, to date little attention has been devoted to clarify the role of Gln-dependent cross talk between macrophages and lymphocytes. In several cell models, the bidirectional transporter SNAT5, coded by SLC38A5, mediates the exchange of Gln among different cell types. Our previous contributions indicated that LPS causes a marked induction of GS in human monocyte-derived macrophages (MDM). Here we verify if, besides synthetizing Gln, LPS-treated MDM also export the amino acid. Moreover, we have also if bacteria induce changes in GS and SNAT5, as well as IFNγ secretion, in autologous co-cultures of MDM and $CD8^+$ T cells and MDM.

METHODS

PBMC-derived human MDM and differentiated THP-1 macrophage-like cells were treated with LPS (1 ng/ml) to assess a) SNAT5 and GS expression by RT-PCR and immunocytochemistry; b) the underlying mechanism; c) 3H-Gln influx/efflux. Co-culture experiments were performed according to the method of Linn et al. (Nature Microbiol, 2022) with $CD14^+$ monocytes and $CD8^+$ lymphocytes isolated through negative immunomagnetic selection. After selection, $CD14^+$ cells were exposed to Bifidobacteria for 2h, $CD8^+$ lymphocytes were added and IFNγ secretion was then determined through ELISA after 48h

RESULTS

In MDM, LPS treatment induced a transient, massive increase of SNAT5 transporter mRNA, an effect that was maximal after 24h, the membrane expression of the transporter protein and an increased 3H-Gln efflux. Enhanced expression of GS was also detected in parallel. SNAT5 and GS induction was confirmed in THP-1 cells, where it was suppressed by the JAK/STAT inhibitor tofacitinib. In $CD14^+$/$CD8^+$ co-cultures, the exposure to selected Bifidobacteria strains caused a marked increase of IFNγ secretion and a significant induction of both GS and SNAT5

CONCLUSIONS

The results recapitulate a model in which, upon activation, macrophages synthesize and extrude Gln, thus ensuring Gln levels in the immune microenvironment permissive for $CD8^+$ activity.

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P126

BLOCKADE OF PD-L1 ENDOCYTOSIS BY UPAR ANTAGONIST PEPTIDES TO IMPROVE CANCER IMMUNOTHERAPY

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BACKGROUND-AIM

Immunotherapy has revolutionized cancer therapy and recent evidences have revealed the clinical efficacy of programmed cell death-1/programmed death ligand-1 (PD-1/PD-L1) antibodies in patients with metastatic breast cancer, melanoma and non-small-cell lung cancer. The therapeutic efficacy of PD-1/PD-L1 inhibitors is high in patients with high PD-L1 expression. Recently, Tseng et collaborators showed that targeting Plasminogen Activator Inhibitor (PAI-1) by its inhibitor tiplaxtinin (TPX) synergizes with anti-PD-L1 checkpoint blockade in a model of murine melanoma, thus paving the way for a more effective melanoma treatment. PAI-1 induced the internalization of surface PD-L1, resulting in the reduction of PD-L1 at membrane level. The membrane-associated plasminogen activation system (urokinase-type plasminogen activator, uPA; uPA receptor, uPAR; uPA inhibitor type-1, PAI-1 is considered one of the main systems involved in tumor invasion and metastasis. Another limiting factor of ICIs is the PD-L1 packaging within specific membrane-enclosed extracellular vesicles (EVs), the so-called exosomal PD-L1. Recent studies have revealed exosomal PD-L1 as a mechanism of tumor immune escape and immunotherapy resistance. We propose to inhibit PDL-1 endocytosis by uPAR inhibitors to maintain high-cell-surface levels of PD-L1 and, at the same time, to reduce the expression of exosomal PD-L1.

METHODS

A375M6 (metastatic melanoma cells) and A549 (non-small cell lung cancer cells) were treated with TPX to evaluate the effect of TPX treatment on exosomal PD-L1 modulation. 2D and 3D cultures from A549 and A375 M6 were treated with TPX and two different uPAR antagonist peptides (WX360 and IPR803).

RESULTS

Our result evidenced that exosomes from TPX-treated A375M6 and A549 show a decrease of exosomal PD-L1 levels, compared to untreated cancer cells. Moreover, we demonstrated that in 2D and 3D cultures of A54and A375 M6 ,TPX and uPAR antagonist peptides are able to block the PD-L1 internalization and, consequently, to increase PD-L1 membrane levels. -

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. In parallel, our results evidenced that the blockade of PD-L1 endocytosis induced a decrease of exosomal PD-L1 levels. These findings have significant implications for immunotherapeutic approaches to cancer therapy.

P127

CD103+ NATURAL KILLER CELLS DISTINCTIVELY INFILTRATE THE TUMORAL EPITHELIUM OF HUMAN LUNG CANCER

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BACKGROUND-AIM

NK cells represent a promising target for cancer immunotherapies. In the present study, we sought to investigate the NK cell compartment infiltrating lung cancer with the aim of providing a deeper understanding of their phenotype and spatial distribution, with particular interest in subsets able to infiltrate tumor nests.

METHODS

We analyzed tumor-infiltrating NK cells from surgical specimens derived from a cohort of 53 Non-Small Cell Lung Cancer (NSCLC) patients. NK cells were analyzed by multicolor flow cytometry for the expression of markers related to tissue-residency, and activation, moreover for functional features such as cytotoxicity and cytokine/chemokine production. Single-cell analyses were paired with assessment of the localization of NK cell subsets in the stroma or tumor nests, in matched FFPE specimens. Finally, the potential of circulating NK cells to infiltrate tumor mass and engender tumor-resident NK cell subsets was assessed further by using a 3D spheroid model of NSCLC.

RESULTS

We showed that a consistent amount of NK cells is able to display an intraepithelial tumor localization (NON-SQK=36 NK cells/mm^2; SQK=7 NK cells/mm^2). This is remarkable since, based on previous findings, NK cells had only been reported to localize in the stroma surrounding tumoral epithelium. This tumor epithelium-infiltrating NK cell subset homogeneously express CD103 integrin and display markers of tissue residency and activation. We found that CD103$^+$ NK cells specifically accumulate in tumor tissues (tumor vs. adjacent lung tissue = 33% vs. 5% of total NK cells), regardless of histotype and the clinical stage. Functionally, they showed dysregulation in primary NK cell functions even if maintaining comparable level of chemokine production. By using a 3D spheroid model of NSCLC, we observed that only circulating CD56^{bright} NK cells, but not the CD56^{dim} counterpart, could efficiently adhere to and infiltrate tumor masses. Interestingly, circulating CD56^{bright} NK cells, which were able to infiltrate in vitro tumor spheroids, acquired CD103 and other features that we observed in NK cells present in the epithelial area of patient's tumor samples.

CONCLUSIONS

Overall, these findings emphasize a unique role of CD103$^+$ tissue-resident NK cells in localizing within epithelial areas of lung tumors, thus supporting the rationale for developing strategies targeting endogenous NK cells to boost antitumor immunity.

P128

OPTIMIZATION OF A GENE PANEL FOR THE STRATIFICATION OF TRIPLE-NEGATIVE BREAST CANCERS (TNBC)

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BACKGROUND-AIM

In 2011, the group of Lehmann proposed the subdivision of TNBC into six molecular subtypes each characterized by potentially new therapeutic targets: 2 forms of basal-like (BL1 and BL2), an immunomodulatory group (IM), a mesenchymal (M), a mesenchymal stem-like (MSL) and a positive subtype for the androgen receptor (Luminal Androgen Receptor, LAR). Objective of this study was to develop and evaluate the feasibility of a gene panel for the classification of TNBC into the 6 molecular classes.

METHODS

RNA was extracted from FFPE sections of 52 TNBC. The characterization of the different molecular subtypes of TNBC was performed by multiplex Real Time qPCR analyzing the following transcripts: PARP1, RAD51, TTK, AURK_A, EGFR, mTOR, MET, EPHA2, PIK3CA, IGF1R, SRC, PDGFRA, mTOR, AR, FGFR4, JAK1 / 2, LYN, IRF1, NFKB, SRC, IGF1R, PIK3CA, NFKB, mTOR, PDGFRa. For the analysis of real-time qPCR data, "gplots" and “stats" packages of the "R" statistical software were used. Hierarchical clustering was performed by Distance “Euclidean” and Hierarchical clustering “Complete linkage”.

RESULTS

Adopting the developed gene panel it was not possible to clearly identify the 6 subgroups. Therefore, we decided to cluster using the "R" software on the basis of genes and samples without seeking correspondences with the 6 expected subgroups. In this way, it was possible to identify 4 subgroups with a gene profile predicting different therapies.

CONCLUSIONS

This preliminary study indicates the feasibility of implementing TNBC diagnostics with gene panels. Further studies are requested to validate the gene panel in a larger case study.

P129

ADVANCING CHRONIC VENOUS ULCER TREATMENT: INSIGHTS FROM RNA SEQUENCING IN AUTOLOGOUS BLOOD THERAPY

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BACKGROUND-AIM

Chronic venous ulcers (CVU) represent a significant clinical challenge due to their slow healing and recurrent nature. Autologous blood therapy has emerged as a promising treatment approach. Clinical studies have demonstrated that autologous blood therapies accelerate wound closure, reduce ulcer size, and improve overall wound healing outcomes. RNA sequencing has been increasingly employed to elucidate the molecular mechanisms underlying the efficacy of autologous blood therapy. By analyzing gene expression profiles through a massive RNA sequencing approach we investigated how autologous blood components modulate biological pathways involved in wound healing processes.

METHODS

The study included 11 subjects affected by CVU. Ulcer biopsies were evaluated by an expert pathologist at two time points (before starting therapy and after 3 months of blood therapy). Transcriptomics was performed on 4 cases and 3 controls, matched for ulcer size. Total RNA was extracted from tissue samples and library preparation was conducted using the FastSelect™ RNA Library Kit (Qiagen). The GeneGlobe platform was employed for data analysis (RAP). Differentially expressed mRNAs were identified using the DESeq2 algorithm, with a significance threshold of the False Discovery Rate (FDR) <0.01. Functional characterization was executed using the Ingenuity Pathway Analysis (IPA) app.

RESULTS

Clinical follow-up revealed a reduction of the ulcer extension and a progressive healing process. The molecular comparison between time points within the experimental group highlighted repair pathways characterized by TGF-β activation. A pro-fibrotic reparative stimulus followed by an hyperproliferative regenerative stimuli characterized the CVU healing processes. Additionally, MAPK and ERK activation and macrophage polarization from M1 to M2 phenotype emerged as additional key points. FOXC1 and ROCK2 appeared to have prognostic positive roles. No differences between cases and controls were highlighted at the first time point.

CONCLUSIONS

Our study supported the positive clinical outcomes of autologous blood therapies and reaffirmed known aspects of wound healing processes highlighting the activation of the described pathways by introduction of blood therapy. Importantly, it underlined the critical roles of signaling pathways and up-regulators, suggesting their potential as future targets for novel therapeutic interventions.

P130

CHARACTERIZATION OF THE GUT MICROBIOTA COMPOSITION OF THE POPULATION RESIDING IN FRIULI-VENEZIA GIULIA THROUGH NEXT-GENERATION SEQUENCING (NGS)

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BACKGROUND-AIM

Gut microbiota is an ecological community of symbiotic and commensal microorganisms with crucial roles in nutrient metabolism, maintaining the structural integrity of the intestinal mucosal barrier, immunomodulation, and pathogen protection. In a healthy adult, the microbiota is dominated by Firmicutes and Bacteroidetes phyla. The composition varies with age, ethnicity, lifestyle, and dietary habits. Given the microbiota's growing role as a modulator of various physiological and pathological conditions, our study aimed to investigate the genetic profile of the microbiome in healthy individuals residing in the Friuli-Venezia Giulia region.

METHODS

Bacterial DNA was extracted from fecal samples obtained from 110 healthy subjects (Qiagen). Analysis of the hypervariable V3-V4 regions of 16S rRNA was performed using Next Generation Sequencing (NGS) on the MiSeq system (Illumina). Data were processed, aligned, and grouped into operational taxonomic units (OTUs). The relative abundance of phyla, classes, orders, families, and species was defined after normalization for the number of 16S gene copies. Correlations between the different microbial populations and the lifestyle of the subjects included in the study were investigated.

RESULTS

We characterized the gut microbiota of 110 subjects, defining normal ranges for phyla, classes, orders, families, genera, and species. Firmicutes was the most represented phylum (51.1%), followed by Bacteroidetes (38.3%) and Actinobacteria (3%). At the class level, Clostridia (45.2%) and Bacteroidia (37.7%) were predominant, while Clostridiales (46.9%), Bacteroidales (26.6%), and Anaeroplasmatales (12.6%) were notable orders. Faecalibacterium prausnitzii (10.3%), Bacteroides vulgatus (4.6%), and Bacteroides dorei (3.5%) being prominent species. Alpha-diversity, enterotype and individual taxa were analyzed. Significant associations emerged between specific genera of microorganisms and age, gender, anti-inflammatory drugs, tobacco consumption and allergies.

CONCLUSIONS

This study provides valuable insights into what constitutes a "healthy intestinal microbiota". The characterization of the microbiota in the Friuli-Venezia Giulia region lays the foundation for future research into regional variations in microbiota composition and its impact on health. Additionally, identifying key microbial markers associated with health and disease states enhances the potential for developing targeted therapies approaches in managing infectious diseases and mitigating antibiotic resistance.

P131

ENHANCING HEALTHCARE-ASSOCIATED INFECTION SURVEILLANCE IN NEONATAL INTENSIVE CARE UNIT (NICU) THROUGH NEXT-GENERATION SEQUENCING (NGS)

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BACKGROUND-AIM

Nosocomial infections in Neonatal Intensive Care Units (NICU) are a significant concern due to the vulnerability of the patient population, primarily consisting of premature and immunocompromised infants. Serratia marcescens is an opportunistic pathogen implicated in nosocomial infections, that are a leading cause of morbidity and mortality. Implementing effective infection control programs in NICU with the integration of Next-Generation Sequencing (NGS) can significantly enhance the detection, monitoring, and prevention of nosocomial infections. Aim of this work was to develop a mapping-based pipeline for creating a phylogeny from bacterial whole genome sequences and for typing strains of nosocomial infections, enabling detailed evolutionary analysis and comparison of microbial genomes.

METHODS

A retrospective study was conducted on 12 strains from 8 patients and 2 strains from environmental swabs of Serratia marcescens, originating from the NICU of the University hospital of Udine, in the period 2023-2024. Genomic DNA was isolated and sequencing libraries were prepared using the FX DNA Library Preparation Kit (Qiagen). Whole genome sequencing (WGS) was performed on an Illumina MiSeq System using a 2 × 300 bp paired-end run. Sequencing files were analyzed with CLC Genomic Workbench (Qiagen) optimizing a specific pipeline for sequence typing.

RESULTS

Data obtained from WGS analysis using the optimized pipeline showed some phylogenetic relationships between strain isolated. In particular, six strains were found to be genetically closely related. Notably, identical genetic compositions were observed in isolates from blood samples, rectal swabs of patients and the environmental swabs, suggesting possible involvement in transmission. We also found a second cluster of infection including another surveillance swab and isolates from the blood and CVC samples.

CONCLUSIONS

The extensive data generated by NGS provides valuable insights into the epidemiology of infections, helping to refine infection control policies and procedures based on real-time evidence.

P132

ROLE OF HEDGEHOG SIGNALING AND KCASH2 IN COLORECTAL CANCER

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BACKGROUND-AIM

The Hedgehog (Hh) pathway is a highly conserved signaling pathway that regulates cell proliferation and differentiation. In the normal intestinal mucosa regulates self-renewal and maintains the stem cell niche. However, its dysregulation is involved in tumor progression of several cancer types, including colon carcinogenesis. Given the dual role of the Hh pathway in colonic mucosa, understanding how this signaling is deregulated may represent an important objective in order to design novel rational therapies. Among the different approaches to inhibit the Hh pathway, the recently characterized family of oncosuppressors Kcash appears to be a promising player. Kcash2 is able to promote Hdac1 degradation, preserving Gli1 acetylation and the suppression of Hh pathway. Thus, we plan to assess the role of Kcash2 in vivo by using newly generated KcashKO mouse and AOM/DSS treatment, definitively linking the Hh signaling overexpression with CRC.

METHODS

The effect of Kcash2 gene deletion in mice was studied using colon tissue from WT and KO mice. Protein and RNA from these tissues were analyzed by Western blot and RT-qPCR to monitor Kcash2 and Hh component levels. Mice were treated with AOM/DSS (10 mg/kg AOM intraperitoneally, then one week of 2% DSS in drinking water). Body weight and clinical symptoms were monitored to assess colitis and cancer progression. Colon tissues from mice sacrificed at specific acute and chronic disease stages were formalin-fixed, paraffin-embedded, and analyzed by standard Hematoxylin/Eosin staining to compare KO and WT mice.

RESULTS

We demonstrated that loss of Kcash2 leads to an increase in Gli1 protein levels and it promotes a hyper activation of Hh-Gli1 activity. In order to induce CRC onset, we used AOM/DSS treatment which confirmed that KO mice are more sensitive to treatment. We observed a higher mortality rate and more marked inflammatory symptoms in KO mice compared to WT. Moreover, we have identified a significantly higher number and size of lesion from KO mice sacrificed at chronic stage of disease. Finally, Kcash2 KO mice resulted more prone to develop dysplasia over extensive area of distal-rectal tract, compared to WT mice.

CONCLUSIONS

Taken together, our evidence indicates that Kcash2 KO mice seem to be more susceptible to AOM/DSS and more prone to develop an extensive dysplastic area, suggesting that Kcash2 could be important for the development of inflammatory bowel disease related CRC.

P133

TAU/MAPT PROTEOSTASIS REGULATES NEUROBLASTOMA RESISTANCE TO DIFFERENTIATION THERAPY

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BACKGROUND-AIM

Retinoic acid (RA) is currently used in clinics to treat neuroblastoma (NB) since it can induce cancer cell differentiation. RA acts by suppressing cancer cell growth through cell cycle arrest, differentiation and apoptosis, but patient survival after treatment is short-term. Tau/MAPT, under the modulation of kinases and phosphatases, is a multifunctional protein involved in the assembly and stabilization of microtubules contributing to the regulation of mitosis in proliferating cancer cells. This study aimed to evaluate whether modulation of Tau protein phosphorylation can influence tumor cell differentiation towards a neuronal-like phenotype, clarifying its potential role as a therapeutic target with a differentiation treatment.

METHODS

SH-SY5Y cell lines were used as reference tumor models and RA as differentiation agent and RA washout was considered as a model mimicking an acquired resistance.

RESULTS

Phosphorylation of Tau protein in NB contributes to cell cycle progression in proliferating cancer cells. In particular, expression analysis in SH-SY5Y cells after synchronization with Nocodazole showed reversible Tau hyperphosphorylation restricted to G2/M phase, in association with a reduction in its microtubules binding affinity. Differentiation treatment induced a phenotypic switch in SH-SY5Y cells from a S-type subpopulation (vimentin-positive) to a N-type subpopulation (vimentin-negative) characterized by neuritic processes, cell cycle arrest in G1/G0 phase, increased expression of neuronal markers including neurofilament-H (NFH) and ß3-tubulin, and spatial and functional reorganization of Tau protein. Cell washout after differentiation revealed an increased expression of phosphorylated Tau after 48h in cells re-entering G2/M phase with respect to RA-differentiated cells. Washed cells were characterized by an increase in vimentin expression and an epithelioid-like morphology, with nearly abortive cytoplasmic processes and large multidotted nuclei. GSK3ß inhibitor, 1-azakenpaullone, was found to reduce phosphorylated Tau expression in both control and differentiated NB cells. In presence of 1-azakenpaullone inhibitor, cell washout failed to increase expression of phosphorylated tau and was associated with a reduction in cancer cell recovery and vimentin-positive cancer cells.

CONCLUSIONS

Therapeutic strategies aimed at interfering with Tau protein proteostasis may counteract the potential resistance effects associated with RA treatment in poorly differentiated neuroblastoma.

P134

SIMULTANEOUS INHIBITION OF MULTIPLE SIGNALING TRANSDUCTION PATHWAYS IN THE TREATMENT OF MEDULLOBLASTOMA

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common form of malignant pediatric brain tumor. MB arise from abnormal proliferation of Cerebellar Granule Precursor Cells (GPCs), with subsequent acquisition of neoplastic phenotype. MB is an heterogeneous group of tumors showing high inter-tumoral histological and transcriptional variability. Current treatment of MB includes surgery, radiotherapy and adjuvant chemoterapy. Despite good prognosis rate, life expetance in patience with relapse is very poor due to acquisition of chemoresistance and metastasization. The aim of this study is to evaluate the effect of three drugs, GANT-61, Afatinib, and Y15 on MB cell lines proliferation, survival and apoptosis. GANT-61 is an inhibitor of Hh-Gli1/2 signaling; Afatinib binds and irreversibly block the kinase activity of ErbB receptor family, while Y15 inhibits the auto-phosporilation of focal-adhesion kinase. In order to develop new possibilities of therapeutic strategies in the treatment of MB, a single-drug approach, as well as multiple drug-approach, will be applied to evalute possible synergistic interactions of these drugs to counteract MB progression.

METHODS

MB cell lines DAOY, ONS-76 and UW-228 cell proliferation has been evaluated by MTT assay following single- and combined drug administration at increasing doses and different time points. Western Blot and RT-qPCR analyses have been performed on treated cell to evaluate the inhibition of the drug targeted pathways as well as the induction of the apoptosis-related genes.

RESULTS

The administration of GANT-61, Afatinib and Y15, alone and in combination, effectively reduces MB cell line-proliferation. Furthermore, we assessed the inhibition of the drug targeted pathway by WB and RT-qPCR analyses. We also detected an increase in the levels of pro-apoptotic genes in the analyzed cell lines when treated with the drugs alone and in combination.

CONCLUSIONS

Medulloblastoma is a pediatric tumor with high frequence of relapse. Tumor re-occurance is often accompanied by acquisition of resistance and has generally a very poor prognosis. Our results demonstrate in vitro that combined inhibition of different signaling pathways has an increased effect in slowing down MB growth and progression, leading to new strategies in the pharmacological treatment of MB.

P135

THE ROLE OF KCASH2 IN THE REGULATION OF THE MITOTIC CHECKPOINT

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BACKGROUND-AIM

MAD2 is the main player in the spindle assembly complex (SAC), essential for chromosomal stability during cell mitosis and preventing defected cellular divisions that may lead to aneuploidy. MAD2 protein levels have been found either aberrantly elevated or reduced in various types of human tumors characterized by chromosomal instability. Recently, it was identified MAD2 as a new interactor of KCASH2, a oncosuppressor identified in our laboratory capable of inhibiting the Hedgehog pathway. Preliminary data demonstrate that KCASH2 induce the ubiquitination and degradation of MAD2, reducing its cellular levels. Characterizing the interaction between KCASH2 and MAD2 could broaden our understanding of the mechanisms involved in regulating MAD2 activity, providing insights into the regulation of the mitotic checkpoint.

METHODS

The KCASH2 ability to bind and downregulate MAD2 protein is evaluated by co-immunoprecipitation and Western Blot assays. The localization of KCASH2 and MAD2 during different phases of the cell cycle is examined by immunofluorescence analysis. Finally, through time-lapse microscopy and FACS analysis we analyzed the cell cycle to observe mitotic progression and identify mitotic defects.

RESULTS

We observed that the silencing or overexpression of KCASH2 affects the cell cycle progression, causing a longer metaphase-anaphase transition compared to KCASH2-WT cells. Moreover, KCASH2 alteration induced mitotic defects, such as chromosome lagging, micronuclei and anaphase bridges. These findings suggest that both silencing and overexpression of KCASH2 affect MAD2 protein levels by altering SAC formation during the cell cycle.

CONCLUSIONS

The interplay between KCASH2 and MAD2 could be implicated in the regulation of assembling of the SAC, influencing the final cell timing of anaphase entry and stringency of chromosome segregation. Therefore the fine characterization of the KCASH2 involvement in the regulation of the mitotic checkpoint could have important implications in the development of new therapeutic approaches directed at the reconstitution of a normal SAC function for the treatment of tumors characterized from chromosomal instability.

P136

TARGETING STING PATHWAY EPIGENETIC ALTERATIONS TO RESTORE INNATE IMMUNE RESPONSE IN NEUROBLASTOMA

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BACKGROUND-AIM

Neuroblastoma (NB), the most common extracranial tumor of childhood, is driven by MYCN amplification (MNA). NBs are refractory to chemo- and immune-therapy due to remarkable heterogeneity and a "cold" immune phenotype associated with low chemokine expression and lack of immune infiltration. Epigenetic and transcriptional profiles of NB tissues reveal two different epigenetic identities, mesenchymal (MES) and adrenergic (ADRN), with higher expression levels of pro-inflammatory cytokines and enhanced immunogenic capability in MES compared to ADRN NB. STING pathway, often inactivated in many tumors by genetic or epigenetic alterations, plays a crucial role in evoking anti-tumor immune response (IR) by converting "cold" tumors into immunogenic ones through stimulation of type I-IFN. Our preliminary data show that STING pathway is impaired in NB cells and primary tumors, particularly in ADRN NBs. Whether STING signaling is involved in MES/ADRN epigenetic switch is to be determined yet.

METHODS

To evaluate epigenetic alterations impeding STING pathway in NBs, pyrosequencing along with an in deep analysis of scRNA-seq data, were carried out. STING pathway activation was assessed by WB analysis and qPCR assay. To investigate whether STING-mediated IR anti-NB cells was evoked upon STING activation, NK cells degranulation in vitro assay was performed.

RESULTS

Reanalyzed scRNA-seq data derived from NB tissues, reveal that cGAS and STING are almost undetectable in ADRN neuroblastic cells, while highly expressed in fibroblasts, endothelial, myeloid and T cells. Conversely, downstream components of the cascade (i.e. TBK1 and IRF3) are expressed at high levels in almost all cell types analyzed. DNA methyltransferase inhibition (DNMTi) restores STING pathway responsiveness to STING agonists or inducers (i.e. cGAS/HT-DNA transfection) in MES/ADRN NB. Finally, cGAS overexpression in MES NB cells can induce innate IR anti-tumor cells through NK activation.

CONCLUSIONS

Our data shed new lights on novel therapeutic approaches based on the restoration of STING pathway by pharmacological inhibition of epigenetic aberrations in NB.

P137

IDENTIFICATION OF CEREBROSPINAL FLUID CYTOKINES EXPRESSION PHENOTYPES IN IMMUNE-MEDIATED NEUROLOGICAL DISORDERS WITH FOCUS ON MULTIPLE SCLEROSIS: APPLICATION OF MACHINE LEARNING ALGORITHMS

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BACKGROUND-AIM

Machine Learning is playing an increasingly important role in healthcare, particularly in diagnostics and therapy. Indeed, it is a promising tool to understand the pathogenetic and diagnostic role of new biomarkers, such as cytokines in immune-mediated neurological diseases. The aim of this study was to identify different cytokine expression phenotypes in a large series of patients with neurological diseases focusing on multiple sclerosis (MS).

METHODS

CSF cytokines (IL-1β, IL-6, IL-8, TNF-α, CXCL10, IFN-γ, IL-10, IL-2Rα, BLC/CXCL13, IL-15 and Fractalkine) and light chain neurofilaments (NfL) were evaluated in a cohort of 141 neurological patients referred to the laboratory of immunopathology. Diagnoses were revised by Neurologists and biomarkers were analysed with customized ultrasensitive multiplex ELISA on Ella instrument (Bio-Techne, USA). using Unsupervised hierarchical agglomerative clustering (HAC) algorithm was executed to identify phenotypic expression clusters.

RESULTS

The HAC identified 9 clusters, three of which presented the best results in terms of numerosity and pathology distribution, since most diseases were spread in these 3 major expression phenotypes, one hyperinflammatory and the other two mildly inflammatory. As concerns MS patients, they were spread in 3 subgroups in which IL-1β, IL-8, CXCL10, IL-15 and Fractalkine were the best discriminative markers. IL-15 has the highest discriminative power in cluster definition and shows the strongest correlation with Fractalkine ($p<0.0001$).

CONCLUSIONS

Clustering analysis identified 3 different MS phenotypes in which IL-15 and fractalkine seem to play major discriminating roles. Data are preliminary but highly promising since these cytokines were already described in MS as concerns cytotoxic brain damage (IL-15) and remyelination (Fractalkine).

P138

CEREBROSPINAL FLUID CYTOKINES SUPPORT THE DIAGNOSIS OF CNS LYMPHOMA: A CASE REPORT

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BACKGROUND-AIM

The diagnosis of cerebral lymphoma can be difficult because initial symptoms are common with many other pathologies and molecular confirmation has a very low sensitivity. The most frequent type of primary central nervous system lymphoma (PCNS) is the diffuse large B-cell one, characterised by constitutive activation and overexpression of STAT3, associated with dysregulation of IL-10 production. The main effect consists of malignant cells survival, proliferation and migration. Although also levels of IL-6 were described to be elevated in PCNS patients, concentrations in cerebrospinal fluid and the degree of STAT3 activation were lower. Therefore, recent studies tried to evaluate IL-10/IL-6 ratio plausibility and performance for diagnosing this rare type of lymphoma. The description of this clinical case aims to demonstrate how cerebrospinal fluid (CSF) dosage of cytokines associated with B-cell proliferation and activity (IL-10 and IL-6) can support an earlier diagnosis.

METHODS

Case report part 1. The story of our patient (female, 71) started in August 2023 with left radicular and lumbosacral pain, followed by similar symptoms in the right lower limb, and left facial nerve paralysis: MRI showed thickening of the left seventh and eighth cranial nerves, interpreted as focal neuritis. after some months she complained right facial paralysis and walking difficulties, voice changes and dysphagia. The first CSF sampling showed atypical lymphocytoid pleocytosis and elevated proteins.

RESULTS

Case report part 2. High levels of IL10 were detected, and with IL-10/IL-6 ratio of 53.8, which raised strong suspicion of lymphomatous pathology. Another brain MRI showed post-contrast enhancement of multiple cranial nerves and solid tissue in the left Meckel's cave. Immunophenotyping highlighted a population with 2% clonal Kappa restriction of lymphocytes. Only after four CSF samplings presence of MyD88 mutation was confirmed.

CONCLUSIONS

In this case report we highlight that CSF dosage of cytokines can support the diagnosis of lymphomatous pathology right from the beginning. The definite diagnosis was possible with genetic analysis after several CSF sampling. In similar cases, would it be possible to establish an early therapy on the basis of the sole cytokine dosage? Further studies are necessary to demonstrate the sensitivity and specificity of the CSF IL10 overexpression and high IL10/IL6 ratio in patients with CNS lymphoma.

P139

INVESTIGATING THE ROLE OF JAGGED LIGANDS IN THE EXTRACELLULAR VESICLES-MEDIATED MODULATION OF MULTIPLE MYELOMA MICROENVIRONMENT

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BACKGROUND-AIM

Multiple myeloma (MM) is a hematological disease whose aggressiveness is mainly due to the pathological interaction with the bone marrow (BM) niche leading to angiogenesis, osteoclastogenesis and immune modulation. The MM aberrant expression of Notch pathway members, Notch2 receptor and Jagged1 and 2 ligands contributes to the supportive behaviour of BM cells leading to the pro-tumor osteoclastogenesis and angiogenesis.
Extracellular vesicles (EV) shed by MM cells are also new key players in the pathological communication with BM microenvironment. Indeed, we already reported the functional effect of Notch2 transferred by MM cell-derived EV (MM-EV) on the BM niche, exploring osteoclastogenesis and angiogenesis. In this work we study the role of Jagged 1 and 2 in the tumorigenic effects of EV.

METHODS

EV were isolated from the MM cell lines RPMI8226 and OPM2 knocked down or not for Jagged1 and 2 (EV SCR and EV J1/2KD). EVSCR and EV J1/2KD were characterized for their ligands expression as well as their ability to activate Notch signaling in recipient cells by an in vivo reporter assay performed on Notch-reporter Tg(T2KTp1bglob:hmgb1-mCherry)jh transgenic zebrafish embryos. The osteoclastogenic and angiogenesic ability of EV SCR and EV J1/2KD was assessed in vitro on RAW264.7 cell line and on human pulmonary artery endothelial cells, respectively. The immune effect was verified on macrophages derived from differentiated monocyte cell line THP1 and on the induction of myeloid derived suppressor cells (MDSC) from peripherical blood human cells.

RESULTS

The results obtained demonstrated that MM-EV increase osteoclast differentiation as well as the angiogenic potential of endothelial cells. Moreover, they modulate cytokine expression profile of macrophages and the number of human MDSC. The presence of Jagged1 and 2 in MM-EV affects their ability to activate Notch signaling in recipient cells and induce their angiogenic, osteoclastogenic and immune effect.

CONCLUSIONS

MM-EV may affect the supportive behaviour of BM cells in a Jagged-dependent way. Thereby targeting Jagged-mediated Notch pathway activation may represent a promising strategy to hamper the pro-tumorigenic activity of MM-EV.

P140

GENE NETWORK ANALYSIS IN MULTIPLE SCLEROSIS PATIENTS INFECTED WITH EPSTEIN-BARR VIRUS TO IDENTIFY BIOMARKERS FOR MONITORING DISEASE EXACERBATION AND PROMPT THERAPEUTIC INTERVENTION

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BACKGROUND-AIM

Multiple sclerosis (MS) is an autoimmune disease characterized by CNS demyelination and neurodegeneration. Recent studies suggest a link between Epstein-Barr Virus (EBV) infection and MS exacerbation. This study investigates gene expression networks in EBV-infected MS patients, comparing stable and active MS phases to find potential EBV-associated biomarkers for disease monitoring therapeutic intervention.

METHODS

Using GEO dataset GSE244312, data from GSM7813411-14 (active MS) and GSM7813417 (stable MS) were processed using GEO2R. Differentially expressed genes were identified between active and stable patients. STRING, Cytoscape and Gephi constructed gene networks, identifying hub genes. Enrichment analysis (Enricher, KEGG) and disease associations (DisGeNet) were examined.

RESULTS

Significant upregulation was observed for genes involved in viral infections defense, dendritic cells regulation, B cell development and interferon production in active MS patients. Pathway analysis links MS to autoimmune diseases such as diabetes mellitus and inflammatory bowel disease. DisGeNet analysis showed associations with classical Hodgkin's lymphoma and lupus.

Genes upregulated in active MS were involved in neuronal development, synaptic signaling, and cation transport, with pathways including ECM receptor interactions, axon guidance and hypertrophic cardiomyopathy. Disease associations included disequilibrium syndrome, acquired and congenital porencephalies.

Conversely, genes downregulated in active MS highlighted a decrease in intermediate filament bundle assembly, Notch signaling and lymphocyte activation.

CONCLUSIONS

This analysis provides indications about molecular underpinnings of EBV-associated MS exacerbation. Upregulated genes in active MS suggest enhanced antiviral response and immune activation, contributing to autoimmune pathology. Downregulation of structural and signaling pathway genes may exacerbate neurological deterioration and immune dysregulation.

This study advances understanding of EBV-related MS mechanisms, identifying potential biomarkers for early therapeutic interventions to mitigate disease progression. Future research will validate identified genes in MS patient PBMCs to develop precise biomarkers for clinical application.

P141

IDENTIFICATION OF NEW VARIANTS AND TRANSCRIPTOMICS ALTERATIONS ASSOCIATED WITH CHEMORESISTANCE IN COLORECTAL CANCER ORGANOIDS

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BACKGROUND-AIM

First-line therapies for colorectal cancer (CRC) are based on combination therapies using 5-fluorouracil (5FU), oxaliplatin (OX) and irinotecan (IRN). Despite a positive initial response, many patients develop drug resistance and a poorer prognosis. The molecular determinants responsible for drug resistance remain still unknown, therefore, this study aims to unveil both transcriptomic and genomic alterations associated with drug resistance by using CRC patient-derived tumor organoids (PDTOs).

METHODS

Drug-sensitive CRC PDTOs were treated with increasing and sublethal doses of 5FU, OX and IRN to generate drug-resistant PDTOs. Whole Exome Sequencing (WES) and transcriptomic analyses were performed to identify genetic variants and differentially expressed genes (DEGs) associated with drug resistance. Bioinformatics investigations were performed using customized pipelines and prediction tools to establish the functional role of altered genes, the SNP density, the ROH and the new variants observed in resistant PDTOs. Confirmatory western blot experiments were also performed.

RESULTS

WES analyses revealed the presence of new variants in the drug-resistant PDTOs with an allele frequency > 10%. Some of these variants are already associated with OX (THSD7B:c.465dup and LARGE1:c.1850T>G), 5FU (DOCK2:c.5262del) and IRN resistance (TYRO3:c.1726G>T) as well as to poor outcomes, Wnt/β-catenin alterations and EMT-mediated drug resistance. RNAseq analyses revealed a total of 2,516, 1,406 and 961 DEGs found in 5FU-, OX- and IRN-resistant PDTOs. These DEGs lead to altered cell proliferation, regulation of rRNA, ncRNA and ribosome dysfunction, kinase activation and protein ubiquitination. All these alterations were driven by MDM2 overexpression which mediated the dysregulation of the MAPK and p53 pathways. H3M2 analyses showed ROH affecting the chr9 in 5-FU and IRN-resistant PDTOs and the chr10 in OX-resistant models. Specific patterns of SNP density were also observed.

CONCLUSIONS

High-throughput profiling of drug-sensitive and drug-resistant PDTOs uncovered the molecular determinants and cellular mechanisms linked to 5FU, OX, and IRN drug resistance paving the way for the development of novel targeted interventions to enhance patients' response.

P142

INTEGRATED ANALYSIS OF DNA METHYLATION AND GENE EXPRESSION IN TCGA TUMORS

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BACKGROUND-AIM

It was recently demonstrated the relationship between DNA methylation (methDNA) and aberrant expression of genes related to cancer hallmarks, including invasion, metastasis, and drug resistance, as well as remodeling of tumor microenvironment and immune escape mechanisms. On these bases, in the present study R-based bioinformatic tools were developed to identify methDNA clusters involved in the regulation of cancer-related genes by analyzing TCGA datasets.

METHODS

Computational analyses were performed to identify adjacent (distance ≤ 200 bp) Illumina CG probesets (CG clusters) considering the UCSC gene region annotation. Then, methDNA status, Beta difference (median Beta value tumor - median Beta value Normal Pool), and correlation values (RNAseq expression of gene and methDNA levels of related CG probesets) were evaluated for the annotated CG clusters in each TCGA tumor type (N = 33) and Normal Pool. Finally, integrated analyses were performed on all identified CG clusters followed by pathway analyses on filtered genes.

RESULTS

The bioinformatic analyses revealed that 147 CG clusters had an opposite methDNA status in at least 20 tumors compared to the Normal Pool. Among the TCGA tumors, DLBC and LGG showed the highest number of exclusively hypermethylated CG clusters (methDNA > 0.6) and Beta difference CG clusters (Betadiff > 0.5 or < -0.5), whereas TGCT and OV displayed most of exclusively hypomethylated CG clusters. Notably, KIRC, LUAD, and MESO showed no significant methDNA variation of CG clusters compared to the Normal Pool. Correlation analysis revealed 38 CG clusters with negative correlation values and 3 positively correlated CG clusters in at least 15 tumors. Such analyses highlighted that most of the hypermethylated intragenic CG clusters were positively correlated with gene expression, while those that were hypomethylated and negatively correlated belonged to the promoter region. Furthermore, integrated analyses identified a subset of genes, including some HLA class I genes, that were strictly regulated by the methDNA status of related CG clusters.

CONCLUSIONS

Overall, the obtained results pave the way for the validation of novel epigenetic biomarkers and potential therapeutic targets for the development of effective anticancer treatments.

P143

THE NEUROPROTECTIVE DRUG EDARAVONE MITIGATES THE MIGRATION OF OVARIAN CANCER CELLS

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BACKGROUND-AIM

Edaravone (EDA), a free radical scavenger, is currently employed to treat amyotrophic lateral sclerosis. However, recent research demonstrated that EDA can have antitumor effects due to its antioxidant and anti-inflammatory properties, however no studies have explored how it affects ovarian cancer.

METHODS

The OVCAR8 cell line was treated with concentrations of EDA ranging from 25 to 1000 μM for 48 hours. MTT and wound healing assays were used to investigate the effects of EDA on cell viability and migration, respectively. The expression of proteins involved in these molecular pathways was determined using Western blot analysis.

RESULTS

No differences in cell viability were found after EDA treatment at different concentrations (25, 50, 100, 200, 400, 700 and 1000 μM) for 48 hours. Conversely, wound healing assays demonstrated that 48h treatment with EDA significantly reduces OVCAR8 cell motility compared to untreated controls ($p < 0.05$). To investigate the molecular mechanisms supervising OVCAR8 migration, the JNK signaling pathway was explored. Western blot analysis revealed that EDA treatment reduced the expression of p-JNK, possibly reflecting the JNK pathway inhibition.

CONCLUSIONS

According to our first findings, EDA can considerably slow migration in the ovarian cancer cell line OVCAR8, but it has no effect on cell viability. However, more extensive research is required to validate the reliability of our results and to further investigate the molecular targets of EDA and its underlying mechanisms in ovarian cancer.

P144

SARS-COV-2-SPECIFIC NEUTRALIZING ACTIVITY AND CYTOKINE PROFILE IN NEWBORNS OF VACCINATED AND/OR INFECTED AND VACCINATED MOTHERS

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BACKGROUND-AIM

Similarly to other pathogens, such as pertussis and influenza, it is important to explore the role of maternal immunization in relation to the risk of acquiring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in infants. Herein, we analysed neonatal protection against SARS-CoV-2 passively acquired after mother vaccination and/or infection (hybrid immunity).

METHODS

Forty newborns of mothers vaccinated with BNT162b2 mRNA vaccine before pregnancy were enrolled in the study. Infants were stratified based on the anamnestic lack/presence of COVID-19 maternal infection: 20 infants from SV (SARS-CoV-2 Vaccinated) mothers and 20 from SIV (SARS-CoV-2 Infected and Vaccinated) women. SARS-CoV-2-specific neutralizing antibody activity (NA) in plasma was assessed by virus neutralization assay (vNTA) against the SARS-CoV-2 Omicron strain (Omi, B.1.1.529) at delivery (T0) and 3 months after birth (T3). As a secondary aim, we also evaluated the immune profile of newborns in terms of plasma cytokine and chemokine production at T0 and T3 by multiplex immunoassay.

RESULTS

At birth, infants of SV mothers displayed significantly lower NA compared to SIV mothers ($p< 0.01$). However, NA declined equally in both groups after delivery at T3. Next, we divided our patients based on the number of immunization events experienced by the mothers, considering both the number of received vaccine doses and the presence of a previous SARS-CoV-2 infection as an immunizing event. Our data confirms that it is not the maternal infection the determining factor, as infants from mothers with less immunization events displayed significantly lower NA compared to newwborns of mothers that experienced more events ($p< 0.01$). Most of plasma cytokines and chemokines showed a trend to higher level at T0 compared to T3 in all 40 newborns. By stratifying our cohort based on a previous infection, a significantly lower concentration of IL-5 and IL-8 (p value <0.05) from T0 to T3 was found in the SIV group only.

CONCLUSIONS

Herein we show that: 1) the number of immunization events confers greater protection at birth; 2) neutralizing activity drops rapidly overtime independently of previous maternal infection, and 3) a proinflammatory profile was observed in all newborns at birth. Our results indicate that, regardless of a previous SARS-CoV-2 infection, a booster dose should be recommended during pregnancy to confer long-lasting protection to the newborn.

P145

SARS-COV-2 NATURAL INFECTION, BUT NOT VACCINE-INDUCED IMMUNITY, ELICITS CROSS-REACTIVE IMMUNITY TO OC43

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BACKGROUND-AIM

The recent SARS-CoV-2 pandemic renewed interest in other previously discovered non-severe acute respiratory syndrome human coronaviruses. Among these, OC43 is a seasonal human coronaviruses widely diffused in the global population (90% seroprevalence in adults), mostly responsible for mild respiratory symptoms. As OC43 protective immunity is short lasting, the aim of this study was to verify if systemic and mucosal SARS-CoV-2 humoral immunity elicited either by natural infection and/or vaccination confers protection against a new OC43 re-infection.

METHODS

Neutralization assayes using plasma and saliva samples of 49 SARS-CoV-2-vaccinated subjects who were never naturally infected and received three doses of BNT162b2 RNA vaccine (SV) and 25 SARS-CoV-2-infected and vaccinated subjects (SIV) were performed against "wild type" SARS-CoV-2 lineage B.1 (EU) and OC43 in VeroE6 cell lines. Sampling was carried out immediately before (T0) and 15 days (T1) post third-dose administration (SV) or 15 days post-infection (SIV). SARS-CoV-2 anti-RDB NAbs were measured, as well, employing a commercial ELISA kit (Viazyme, Delft, Netherlands). Analyses on saliva at T1 were performed on a subset of SV (n = 18) and SIV (n = 15).

RESULTS

SARS-CoV-2-specific neutralizing activity (NA) significantly increased after third vaccine dose administration in plasma ($p<0.0001$) and saliva ($p<0.01$) from SV; however, this NA was not protective against OC43. Conversely, SARS-CoV-2 NA triggered by natural infection in plasma and saliva of SIV proved to be cross-reactive and protective against OC43 in both plasma ($p<0.05$) and saliva samples ($p<0.05$). A statistically significant difference was further confirmed by the anti-RBD Nabs assay in saliva samples at T1 ($p<0.001$). Indeed, the SIV group showed higher levels than the SV group.

CONCLUSIONS

Overall, this study on immunity to SARS-CoV-2 suggests that compared to vaccine-induced immunity natural infection elicits a broader and cross-reactive immunity, which results in protection from viruses sharing sequence homology, at both systemic and mucosal level. As the oral cavity represents the main entry route for coronaviruses, these results support the development of a pan-coronavirus vaccine to prevent new infections and re-infections.

P146

DISCOVERY OF A NEW SELECTIVE INHIBITOR OF ENDOPLASMIC RETICULUM AMINOPEPTIDASE 1 FOR TARGETING HEDGEHOG-DEPENDENT CANCERS

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BACKGROUND-AIM

Inappropriate 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. SHH medulloblastoma subgroup (SHH-MB), is extremely heterogenous and shows resistance to current treatments. Understanding the molecular mechanisms that regulate this pathway is crucial for the identification of new therapeutic targets. Previously, we identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1), a key player of the immune response, as a new positive regulator of the SHH pathway and promising therapeutic target for SHH-MB. However, the lack of selective inhibitors for ERAP1 limits its therapeutic potential.

METHODS

Compound N1, an alkaloid, was selected by virtual screening of a library of natural products against crystallographic structure of the catalytic domain of ERAP1. The efficacy of N1 to inhibit ERAP1 activity has been 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/USP47/ βTrCP regulatory axis was assessed by in vitro pull-down and co-IP assays. Finally, the anticancer efficacy of N1 was investigated in preclinical heterotopic and orthotopic allograft model of SHH-MB also evaluating its ability to cross the the blood-brain barrier by ultra-high-performance liquid chromatography coupled with Electrospray Mass Spectrometry (HPLC/MS) of N1 treated mice cerebella.

RESULTS

In this study, we identified compound N1 as a new selective, potent and non-toxic inhibitor of ERAP1. We demonstrated that N1 directly binds to ERAP1, blocking its function and impairing the association of ERAP1 with USP47. This event promotes βTrCP protein stability and Gli1 degradation, thereby counteracting SHH signaling and SHH-dependent cell proliferation. Remarkably, we showed that N1 crosses the blood-brain barrier and suppresses SHH-MB growth both in vitro and in vivo.

CONCLUSIONS

Our finding strongly indicates N1 as a good candidate for further preclinical studies in the treatment of SHH-dependent tumors.

P147

THE INTERPLAY AMONG WNT/β-CATENIN FAMILY MEMBERS IN COLORECTAL ADENOMAS AND SURROUNDING TISSUES

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BACKGROUND-AIM

Colorectal adenoma undergoes neoplastic progression via the normal epithelium-adenoma-adenocarcinoma sequence as reported in the Vogelgram. The risk of developing cancer is strongly associated with the number and size of adenoma and with the subtype. Currently, adenomatous polyps can be distinguished on the basis of histology: the prevalence are tubular, 5-15% are villous and tubular/villous. Considering the increased risk for malignant transformation described for tubular/villous adenomas, patients diagnosed with adenomatous polyposis are at increased risk of developing CRC. The Wnt/β-catenin pathway plays a key role in the onset of colorectal adenoma, in particular intestinal cells first acquire loss-of-function mutations in APC gene that induce the formation of adenomas.

METHODS

Wnt/β-catenin pathway APC, Wnt3a, Wnt5a, LEF1,BCL9 genes and protein expression analyses were conducted by qRT-PCR and western blot in 68 colonic samples (polyps and adjacent mucosa) from 41 patients, of which 17 affected by FAP. Ten normal colonic mucosal samples were collected from 10 healthy donors.

RESULTS

In this study both APC gene and protein resulted less expressed in colon tumor compared to the adjacent colonic mucosa. Conversely, activated β-catenin was more expressed in polyps than in the adjacent mucosa. All results confirmed literature data on carcinomas. A statistically significant correlation between Wnt3a and BCL9 both in polyps and in the adjacent mucosa underlies that the canonical Wnt pathway is activated in early colon carcinogenesis and that the adjacent mucosa is already altered.

CONCLUSIONS

This is the first study analyzing the difference in expression of Wnt/β-catenin pathway in human colorectal adenomas. Understanding the progression from adenomas to colorectal carcinomas is essential for the development of new therapeutic strategies and improving clinical outcomes.

P148

HUMAN MULTILINEAGE 3D SPHEROIDS UNVEIL THE ROLE OF LGALS3 IN STEATOTIC LIVER DISEASE AND FIBROSIS

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BACKGROUND-AIM

Galectin 3 (Gal-3), a member of the galectin family, plays a crucial role in numerous physiological and pathological processes, including the metabolic dysfunction-associated steatotic liver disease (MASLD). Although the approval of the first pharmacological therapy for MASLD, the identification of molecular pathways and targets to improve the diagnosis and the outcome of the disease remains crucial. Elevated serum levels of Galectin 3 have been observed in patients with steatohepatitis and cirrhosis, suggesting its role as a potential pharmacological target. In this study, we investigated the role of Galectin 3 in MASLD, by employing a 3D in vitro model of steatosis.

METHODS

HepG2 and LX2 were seeded, in the ratio of 24:1, to form a 3D spheroid. The cells underwent incubation with either LGALS3 siRNA or scramble (SCR), immediately following seeding. After 48 hours, cells were treated with a combination of palmitic and oleic acid (PAOA 1:2). 96 hours after seeding, spheroids were collected, and lipid accumulation and collagen production were analysed through Oil Red-O (ORO) staining and collagen 1-alpha-1 immunofluorescence, respectively.

RESULTS

Silencing LGALS3 significantly diminished lipid and collagen accumulation without affecting cells viability. Genes involved in lipid biosynthesis and storage, transport, and β-oxidation were analysed. The most intriguing result was observed in β-oxidation, where the genes CPT1A and PPARG, show down regulation in the spheroids silenced for LGALS3.

CONCLUSIONS

Obtained results suggest that Galectin 3 is involved in the regulation of fatty acid and collagen accumulation, and it might represent a promising target for MASLD patients. Finally, 3D spheroids may represent an in vitro model to study the role of Galectin 3 in MASLD.

P149

DEFINING THE ROLE OF ACP2 IN THE REGULATION OF ERAP1 FUNCTION IN HEDGEHOG MEDULLOBLASTOMA

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BACKGROUND-AIM

The Sonic Hedgehog (SHH) signaling plays a crucial role during embryonic development and its aberrant activation has been found in several human cancers, including medulloblastoma (MB), the most common and aggressive pediatric brain tumor. The molecular heterogeneity of SHH-MB and the occurrence of tumor relapse after standard treatments limits the effectiveness of current therapies making the SHH-MB a challenging disease to treat.
Recently we identified the endoplasmic reticulum aminopeptidase 1 (ERAP1), a key player of the immune response, as a positive regulator of SHH signaling acting as activator of GLI transcription factors, the final effectors of the pathway. This study unveiled a non-canonical function of ERAP1 in cancer development that deserves to be further investigated.

METHODS

By mass spectrometry analysis we identified the acid phosphatase 2 (ACP2), a soluble luminal hydrolase, as a new ERAP1 binding partner. ACP2 is involved in mouse cerebellum development and Acp2-/- mice show cerebellar hypoplasia with reduced granule cells and cerebellar disfunction. Biochemical assays were used to confirm ACP2/ERAP1 binding and characterize the impact of ACP2 modulation on ERAP1 and GLI1 proteins. Antigen presentation assays were used to evaluate ERAP1 enzymatic activity in the presence of ACP2. The biological relevance of ACP2 was assessed in cerebellar granule cells and murine SHH-MB cells, both in vitro and in vivo.

RESULTS

Endogenously ACP2 binds ERAP1 in SHH-dependent cells. ACP2 counteracts ERAP1 enzymatic activity impairing its protein stability and acts as a negative regulator of SHH signaling. Therefore, increasing amounts of ACP2 significantly reduce GLI1 transcriptional activity in a dose-dependent manner. Biologically, ectopic expression of ACP2 inhibits the proliferation of murine granule neuron progenitors (GNPs), the cells of origin of SHH-MB, and primary murine SHH-MB cells both in vitro and in vivo.

CONCLUSIONS

Our findings identify ACP2 as an antagonist of the SHH pathway which alters ERAP1 protein stability. Further investigations are needed to assess how ACP2 mediates ERAP1 regulation. The characterization of ACP2/ERAP1/GLI1 axis in the control of SHH signaling may offer insights to efficiently target ERAP1 in SHH-MB

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MIR-214-INDUCED MELANOMA HYPERPIGMENTATION AND THERAPY RESISTANCE: MOLECULAR INSIGHTS

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BACKGROUND-AIM

Despite the last decade having witnessed a revolutionary benefit in the care of cutaneous melanoma (CM) patients with the advent of immune-checkpoint inhibitors (ICI), resistance onset urges the development of innovative strategies. Melanin affects the course of melanoma, being hyperpigmentation related to therapy resistance, ICI included. Despite the main melanin function being protecting against damage from light by scavenging reactive oxygen species (ROS), melanin can become a photosensitizer/pro-oxidative agent itself depending on melanin type and redox intracellular state. The melanin ROS-scavenging activity mainly relies on its chelating activity of metal ions including iron, which indeed induces melanogenesis because of its high ROS-generating activity. In the context of melanoma hyperpigmentation and therapy resistance, experimental data suggest a crucial role of miR-214 and this study aims to elucidate the interplay among miR-214, ROS, and iron in hyperpigmented/resistant CM, and eventually identify molecular targets to restore therapy response.

METHODS

Melanoma cells were forced to stably overexpress miR-214 (miR-214+) through the PiggyBac transposon system. Intracellular melanin content was quantified by spectrophotometry, melanosomes observed at the transmission electron microscope, and key melanogenic proteins by western blot (WB). ROS were detected in flow cytometry (FC), and the intracellular content of iron was quantified by colourimetric assay. The therapy response of melanoma cells was assessed in 2D/3D colourimetric, luminescent, FC, and colony assays. miR-214 levels in plasma samples of CM patients treated with ICI at the Careggi University Hospital in Florence were quantified by droplet digital PCR.

RESULTS

miR-214+ melanoma cells showed increased pigmentation together with deregulated iron metabolism, increased ROS, and a reduced Glutathione S-transferase Zeta 1 (GSTZ1) expression, an anti-oxidant protein also involved in the catabolism of the melanin precursors phenylalanine and tyrosine. miR-214+ hyperpigmented melanoma cells showed less responsiveness to chemo-, target, and radiotherapy in vitro than control. Higher levels of miR-214 were found in plasma samples of ICI-treated non-responder CM patients compared to responders.

CONCLUSIONS

miR-214 induces the development of hyperpigmented, resistant melanoma cells. A deeper molecular/mechanistic view could be crucial to identify new targets to restore therapy sensitivity in non-responder CM patients.

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BREAKING CHEMOTHERAPY RESISTANCE IN GASTRIC ADENOCARCINOMA: IMMUNOGENIC CELL DEATH INDUCTION BY CARBONIC ANHYDRASE IX TARGETING

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BACKGROUND-AIM

Gastric cancer (GC) is the fifth most common malignancy worldwide and the fourth leading cause of cancer-related death. When tumor resection is not possible, the perioperative chemotherapy (pCT) FLOT (Leucovorin,5-Fluouracil, Docetaxel, and Oxaliplatin) represents the standard of care, at least in Europe, to enhance patient overall survival. However, chemoresistance onset inevitably hampers treatment efficacy. Recently, we identified carbonic anhydrase IX (CAIX) as a promising target in GC patients, as its expression was correlated with resistance to the pCT regimen. Moreover, pre-clinical evidence showed that CAIX inhibition by the SLC-0111 compound - currently under phase Ib clinical trial for metastatic ductal pancreatic cancer – allowed boosted therapy response even in resistant GC cells. Our ongoing study aims to explore the mechanisms behind SLC-0111-induced cytotoxicity in GC and its ability to induce immunogenic cell death, thereby potentially triggering a broad anti-cancer immune response.

METHODS

SLC-0111 and FLOT were administered as mono- or combined therapies to sensitive and FLOT-resistant GC cell lines. Cell death pathways and Damage Associated Molecular Patterns (DAMPs) expression by dying GC cells were assessed through flow cytometry, ELISA, and luminometry. The phenotype of immune cells exposed to dying GC cells was evaluated in vitro by qPCR, WB, and ELISA.

RESULTS

Apoptotic and non-apoptotic immunogenic cell deaths such as alkaliptosis and ferroptosis were observed in GC subjected to the SLC-0111/FLOT treatment. Analysis of DAMPs showed increased exposure of Calreticulin, and elevated release of Annexin A1, High Mobility Group Box 1, and ATP by GC cells treated with SLC-0111/FLOT compared to the control group. Macrophage exposure to such a DAMP-enriched microenvironment resulted in M1 activation.

CONCLUSIONS

In summary, our findings suggest that the SLC-0111/FLOT combination therapy not only improves treatment effectiveness and restores sensitivity in resistant gastric cancer cells but also has the potential to induce immunogenic cell death, potentially triggering an anti-cancer immune response to combat tumor progression.

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DYNAMIC REGULATION OF HISTONE LACTYLATION AND ACETYLATION IN PDAC: IMPACT OF OXAMATE AND SAHA THERAPIES

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BACKGROUND-AIM

Metabolic reprogramming and epigenetic alterations contribute to the aggressiveness of pancreatic ductal adenocarcinoma (PDAC). Like Acetylation, lactate-dependent histone modification is a new type of histone mark, which links glycolysis metabolite to the epigenetic process of lactylation (Kla). In addition, Histone lactylation and acetylation compete for epigenetic modification of lysines and mark the levels of lactates and acetyl-CoA. Our research sought to elucidate the intricate molecular interplay between histone lactylation and acetylation in PDAC system, their competition for lysine residues, and the potential implications of these epigenetic modifications in cellular fate, particularly in the glycolytic regulation.

METHODS

Lactate concentration and pan-Kla levels were assessed in low and high glycolytic phenotype system (PL-45 and Mia PaCa II) and were subjected to both lactate dehydrogenase A (LDHA) inhibitor (oxamate) and Pan-HDAC inhibitor SAHA. Western blot and LDH cytotoxicity assay was performed to evaluate the time frame regulation and rebalancing phenomenon on both lactylation and acetylation residues upon Oxamate and SAHA treatment.

RESULTS

This study provide insights into the regulatory mechanisms of lactylation and acetylation in the potential therapeutic implications of targeting LDHA and HDACs in PDAC metabolism. Time-dependent regulation and potential rebalancing effects on lactylation and acetylation residues were observed. As with oxamate reduction in the several lactylated residues were observed after 24h and then recompensate after 48hr's upon oxamate treatment. In parallel upon SAHA treatment both lactylation and acetylation were increased in a time frame of 24 to 48hrs. The amount of lactate released also differed depending on the type of treatment, since with oxamate the lactate level was reduced, but then increased, whereas with SAHA the level of lactate increased from 24 to 48 hours after treatment.

CONCLUSIONS

In summary, this study provides valuable insights into the complex regulation of histone lactylation and acetylation in PDAC and underscores the importance of considering the temporal dynamics of these modifications when developing targeted therapies that aim to modulate PDAC metabolism.

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THE CYTOSKELETON REGULATOR INVERTED FORMIN INF2 REGULATES THE SHH PATHWAY AND IS INVOLVED IN MEDULLOBLASTOMA TUMORIGENESIS

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BACKGROUND-AIM

Medulloblastoma (MB) is a lethal pediatric malignancy of the cerebellum. The high heterogeneity of MB makes extremely difficult determining a successful therapy. Among MB's molecular subgroups, Sonic Hedgehog (SHH) is the most abundant and it is characterized by alterations of key components of the SHH development pathway. However, the molecular mechanisms driving SHH-MB require to further be unveiled to design more effective therapies. Here, we identified INF2, a formin involved in the regulation of actin and microtubule cytoskeletal dynamics, as a putative negative regulator of SHH signaling and SHH-dependent MB growth.

METHODS

Luciferase functional assays, western-blot and RT-qPCR have been performed to analyze the effect of INF2 on GLI1 (the final effector of SHH signaling) transcriptional activity and expression. Proliferation assays and Brillouin microscopy have been carried out on primary murine SHH-MB cells to test the effect of INF2 modulation on SHH-MB growth and stiffness.

RESULTS

We demonstrated that INF2 is a negative regulator of SHH signaling, with an opposite role previously described for mDia formin. The overexpression of INF2 counteracts the positive effects of mDia on GLI1 transcriptional activity and expression. Moreover, INF2 is highly expressed in late stages of murine cerebellum development when SHH signaling is switched off, showing an opposite trend to mDia. Accordingly, the INF2 genetic silencing increases the expression of GLI1 and the proliferation of the granule neuronal progenitors (GNPs), the cells of origin of MB. Interestingly, INF2 protein levels were strongly reduced in SHH-MB samples, contrarily to mDia. Notably, the overexpression of INF2 in SHH-MB primary cells from Math1-Cre/Ptc1fl/fl mice significantly inhibits the cell proliferation as consequence of the reduction of GLI1 expression levels and increases the stiffness of primary SHH-MB cells, suggesting that INF2 could affect tumor cell motility and invasiveness.

CONCLUSIONS

Overall, our findings unveil INF2 as new player of the SHH pathway and illuminate on the role of cytoskeleton in SHH-MB for the design of innovative interventions.

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DEFINING THE ROLE OF ACP2 IN THE REGULATION OF ERAP1 FUNCTION IN HEDGEHOG MEDULLOBLASTOMA

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BACKGROUND-AIM

The Sonic Hedgehog (SHH) signaling plays a crucial role during embryonic development and its aberrant activation has been found in several human cancers, including medulloblastoma (MB), the most common and aggressive pediatric brain tumor. The molecular heterogeneity of SHH-MB and the occurrence of tumor relapse after standard treatments limits the effectiveness of current therapies making the SHH-MB a challenging disease to treat.
Recently we identified the endoplasmic reticulum aminopeptidase 1 (ERAP1), a key player of the immune response, as a positive regulator of SHH signaling acting as activator of GLI transcription factors, the final effectors of the pathway. This study unveiled a non-canonical function of ERAP1 in cancer development that deserves to be further investigated.

METHODS

By mass spectrometry analysis we identified the acid phosphatase 2 (ACP2), a soluble luminal hydrolase, as a new ERAP1 binding partner. ACP2 is involved in mouse cerebellum development and Acp2-/- mice show cerebellar hypoplasia with reduced granule cells and cerebellar disfunction. Biochemical assays were used to confirm ACP2/ERAP1 binding and characterize the impact of ACP2 modulation on ERAP1 and GLI1 proteins. Antigen presentation assays were used to evaluate ERAP1 enzymatic activity in the presence of ACP2. The biological relevance of ACP2 was assessed in cerebellar granule cells and murine SHH-MB cells, both in vitro and in vivo.

RESULTS

Endogenously ACP2 binds ERAP1 in SHH-dependent cells. ACP2 counteracts ERAP1 enzymatic activity impairing its protein stability and acts as a negative regulator of SHH signaling. Therefore, increasing amounts of ACP2 significantly reduce GLI1 transcriptional activity in a dose-dependent manner. Biologically, ectopic expression of ACP2 inhibits the proliferation of murine granule neuron progenitors (GNPs), the cells of origin of SHH-MB, and primary murine SHH-MB cells both in vitro and in vivo.

CONCLUSIONS

Our findings identify ACP2 as an antagonist of the SHH pathway which alters ERAP1 protein stability. Further investigations are needed to assess how ACP2 mediates ERAP1 regulation. The characterization of ACP2/ERAP1/GLI1 axis in the control of SHH signaling may offer insights to efficiently target ERAP1 in SHH-MB.

P155

THE REPURPOSED DRUG NITROXOLINE ENHANCES THE EFFECTIVENESS OF GEMCITABINE IN REDUCING TUMOR GROWTH AND LUNG METASTASES IN MOUSE MODELS OF PANCREATIC CANCER

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BACKGROUND-AIM

Pancreatic cancer (PC) exhibits limited or no response to current therapies, highlighting the urgent need for more effective treatments. Previous research identified nitroxoline (Nitro), a non-anticancer agent, as a promising repurposed drug for treating PC. This study evaluates the impact of Nitro in combination with gemcitabine (Gem) on PC cell viability. Additionally, we investigated the efficacy of Nitro and Gem, both as single agents and in combination, in PC xenograft mouse models.

METHODS

Cytotoxic effects of Nitro and Gem, as single agents or in combination, on PC cells were assessed by MTT. Subsequently, the antitumor potential of the drug combination was evaluated in immunodeficient NSG mice, subcutaneously xenografted with three human PC cell lines (KP-4, PATU-8902, and AsPC-1). Mice were divided into four treatment groups: 1) vehicle, 2) Nitro, 3) Gem, and 4) combined Nitro and Gem. At the end of experiments, mice were euthanized, and both organs and tumors were fixed and embedded for histological and immunohistochemical analysis.

RESULTS

Combinations of Nitro and Gem affected PC cell viability in vitro in a dose-dependent manner. Their effect exceeded those of single agents at the highest concentrations tested. In KP-4 and PATU-8902 xenograft models, the combined treatment achieved a tumor growth inhibition more pronounced than, or comparable to, single agents. In AsPC-1 xenograft model, the combination of Nitro and Gem did not reduce tumor growth, as compared to single agents. Notably, the combination of drugs decreased lung metastases in all xenograft models, as compared to single treatments, even in models where the effect on primary tumor growth was less pronounced.

CONCLUSIONS

We demonstrate for the first time that combining Nitro with Gem restricts metastatic disease in PC. These findings suggest that repurposed drug Nitro could be profitably added to Gem to expand therapeutic options for PC.

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CRUCIAL ROLE OF GM-CSF IN HUMAN AUTOIMMUNE GASTRITIS.

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BACKGROUND-AIM

Autoimmune gastritis (AIG) is characterized by an inflammatory infiltrate in the gastric mucosa and loss of parietal cells. The major autoantigen in AIG is the gastric proton pump H+/K+-adenosine triphosphatase (H+/K+-ATPase) of parietal cells. It has been shown that GM-CSF is an important factor for the development of experimental AIG.
Our work aimed to investigate GM-CSF in the gastric mucosa of patients with AIG and serum levels of patients with AIG and pernicious anemia (PA), in healthy subjects (HC), and in patients with iron deficient anemia (IDA) without AIG.

METHODS

We investigated the serum levels of GM-CSF in 43 patients with AIG and PA, in 20 patients with IDA and no autoimmune gastritis, and in 47 HC. Furthermore, we analyzed the GM-CSF production by gastric lamina propria mononuclear cells (LPMC) in 8 patients with AIG/PA and 4 HC.

RESULTS

We found that patients with AIG/PA have significantly higher serum levels of GM-CSF (112.66 ± 32.36 pg/ml) than patients with IDA (98.7 ± 97.6 pg/ml; p=0.024) and healthy subjects (77.11 ± 13.74 pg/ml; p<0.01). Gastric LPMC from all AIG/PA patients were able to produce significantly higher levels of GM-CSF (415.77 ± 54.52 pg/ml) than HC (46.71 ± 7.8 pg/ml) (p<0.01).

CONCLUSIONS

Altogether, our results indicate that GM-CSF serum levels are significantly increased in patients with AIG/PA but not with IDA and that GM-CSF is produced in vivo in the stomach of AIG/PA patients. These data open a new perspective on the pathogenesis of human AIG/PA and suggest that GM-CSF may represent a novel important tool for the management of patients with AIG/PA.

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ADIPOCYTE-DERIVED EXTRACELLULAR VESICLES SUSTAIN MITOCHONDRIAL METABOLISM IN BREAST CANCER CELLS: A NEW LINK BETWEEN OBESITY AND BREAST CANCER BIOLOGY

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BACKGROUND-AIM

Obesity has been strongly associated with breast cancer (BC) risk and progression. Nevertheless, a substantial knowledge gap regarding the relationship between adipocytes and BC biology still remains to be covered. In addition to the deregulated levels of hormones, lipid metabolites, inflammatory cytokines and adipokines, obese adipose tissue dysfunction is associated with an enhanced release of extracellular vesicles (EVs), important mediators of intercellular communication. We previously showed that adipocyte-derived EVs exert a profound influence on BC growth and progression through Hypoxia-inducible factor 1 alpha (HIF-1α) activity. Here, to gain further insights into the role of EVs in breast adiponcosis, we analyzed the effects of adipocyte-derived EVs on the BC proteome.

METHODS

EVs were isolated by ultracentrifugation from 3T3-L1 adipocytes (3T3-L1A-EVs) and fully characterized following MISEV guidelines. We employed MCF-7/BT-474/ZR-75 BC cells. Mass spectrometry (MS) and bioinformatic tools were used to analyze the BC cell proteome. Seahorse/ATP production/Mitotracker/OXPHOS expression analyses were performed to investigate EV impact on BC cell metabolism. Genetic/pharmacological approaches were used to inhibit HIF-1α activity.

RESULTS

A total of 4411 proteins were identified by MS, of which 39 and 59 were significantly up- and down-regulated in BC cells treated with 3T3-L1A EVs. Bioinformatic analyses revealed the involvement of these de-regulated proteins in cell metabolism. Accordingly, we found an increase in mitochondrial activity and respiration along with an enhanced ATP production in EV-treated BC cells. Of note, silencing HIF-1α or using the HIF-1α inhibitor KC7F2 counteracted the 3T3-L1A-EV-mediated effects on BC cell metabolism.

CONCLUSIONS

These data provide new evidence in the obesity-BC link, highlighting a potential role of adipocyte-derived EVs in shifting cell metabolism towards oxidative phosphorylation. A better understanding of these mechanisms could provide specific biomarkers/innovative targets (i.e. HIF-1α) that may allow a personalized management of obese BC patients.

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CHEMATSUSTAIN: IMPLEMENTING INNOVATIVE METHODS FOR SAFETY AND SUSTAINABILITY ASSESSMENTS OF CHEMICALS AND MATERIALS PARTICULARLY AT NANO LEVEL IN THE EUROPEAN UNION

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BACKGROUND-AIM

CheMatSustain recognizes the transformative potential of Chemicals and NanoMaterials (CNMs) and the significance of Safe and Sustainable by Design (SSbD) strategies to reduce their impact to health and environment.

METHODS

Identification and selection of CNMs; in vitro models; CNMs high-resolution analysis; delivery of data with computational modelling; evaluating the SSbD; development of harmonized standardized test methods for regulatory contexts; enhancement of the efficiency and effectiveness in CNMs development; fostering the uptake of the new methods among academia, public authorities and the private sector.

RESULTS

CheMatSustain aims to enhance and standardize screening and testing methods, protocols, and assessments for CNMs within the EU. The objective is to create a set of tools that are robust, reliable and efficient to improve the safety and sustainability of these substances.

CONCLUSIONS

With a commitment to shape a sustainable future and align with the SDG's and the EU Green Deal, our mission is to lead the way in pioneering innovative methods for safety and sustainability assessments of chemicals and materials across the EU. Join us in our journey towards a safer, more sustainable future (https://chematsustain.eu/).
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PATHOGENIC ROLE OF THE R200W VHL MUTATION IN CAROTID BODY PARAGANGLIOMA: A CASE STUDY AND POPULATION IMPLICATIONS

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BACKGROUND-AIM

The phenotypic implications of rare mutations in genes related to hereditary head and neck paraganglioma (HNPGL) are not well understood. The R200W germline mutation in the VHL gene, linked to Chuvash polycythemia in homozygotes, leaves heterozygous carriers disease-free. However, we report a case of a heterozygous patient for the R200W germline VHL mutation who developed a carotid body paraganglioma, showing a strong allelic imbalance towards the mutated C598T allele due to somatic loss of heterozygosity (LOH).

METHODS

The mutation was identified through next-generation sequencing (NGS) of 81 oncogenes and tumor suppressor genes and validated via Sanger sequencing. Immunohistochemistry (IHC) for hypoxia-inducible factors (HIFs) and real-time RT-qPCR for 11 target genes were performed on the tumor and paired normal tissues to evaluate transcriptional activation. Additionally, PCR-RFLP using the restriction enzyme Fnu4HI was conducted on 60 HNPGL cases to check for the Chuvash mutation.

RESULTS

IHC showed nuclear immunostaining for HIF2A in 45% of tumor cells, while HIF1A staining was rare. Gene expression analysis revealed upregulation of HIF2A target genes, including the ADO-regulated N-degron substrates RGS4 and RGS5, associated with hypoxia adaptation. Atypical expression of RGS4 and RGS5 was found in the neuroepithelial component of the carotid body paraganglioma.

CONCLUSIONS

HIF2A is the primary HIF isoform activated by VHL inactivation in this Chuvash mutation-related HNPGL. HIF2A-mediated pathway activation, supported by IHC and gene expression analyses, suggests a pathogenic role for the VHL R200W mutation. Our findings highlight the importance of monitoring populations with recurrent Chuvash mutations due to founder effects, such as in central Russia and the island of Ischia.

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CALORIE RESTRICTION AS A NOVEL THERAPEUTIC TOOL TO MODULATE IMMUNE SYSTEM DURING MULTIPLE SCLEROSIS

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BACKGROUND-AIM

There is a strong relationship between metabolic state and immune tolerance through a direct control exerted on immune cells by specific intracellular nutrient-energy sensors. An increased "metabolic work load" represents a novel issue linking metabolism with loss of self-immune tolerance. In this context, several dietary interventions have been shown to influence disease progression of experimental autoimmune encephalomyelitis (EAE), the experimental model of Multiple Sclerosis. Our approach aims at dissecting at the cellular level the mechanism of action of Caloric Restriction (CR) on disease progression, in relapsing remitting Multiple Sclerosis (RR-MS) subjects.

METHODS

Firstly, we examine the impact of CR on nutritional status and on the immunophenotype of different circulating immune cells of RR-MS subjects. We also investigated the effect of different dietary regimens on the metabolic asset of conventional T (Tconv) cells (measurement of glycolysis) and on the ability to induce tolerogenic regulatory T (Treg) cells from RR-MS subjects.

RESULTS

We observed that CR modulates the activation of different immune T cell subsets. Moreover, CR is able to reduce glycolytic capacity of pro-inflammatory T cells and promote peripheral conversion of inducible Treg cells compared to Free Diet (FD)-RR-MS subjects.

CONCLUSIONS

Overall, these data suggest that modulation of metabolic state via calorie restriction is able to improve the outcome of RR-MS and efficacy of first line drug treatment.

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RHYTHMIC METABOLIC ADAPTATIONS TO OPPORTUNISTIC INFECTIONS

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BACKGROUND-AIM

Circadian clocks evolved to coordinate rhythms in behaviour and physiology around the 24 hour. In mammals, circadian rhythms and metabolism are highly intertwined. The clock machinery controls rhythmic levels of circulating metabolites, as well as rate-limiting enzymes catalyzing their biosynthesis or degradation. During infections, major metabolic adaptation occurs in mammalian hosts and intersects with those of pathogens and the microbiota. The circadian regulation of the availability of nutrients is therefore a fundamental aspect that may influence the outcome of infections. Among nutrients, the amino acid tryptophan (trp) and its downstream metabolites are crucial regulators of both immunity and the circadian clock. Trp is metabolized through three different pathways: the host kynurenine (kyn) and serotonin pathway, driven by the enzymes Ido1 and Tph1, respectively, and the microbial indole pathway, activating the transcription factor AhR. A circadian regulation of the kyn pathway in the host response against Aspergillus fumigatus opportunistic infection has been recently demonstrated.

METHODS

We performed circadian in vivo experiments in mice genetically deficient for Ido1, Tph1 and AhR and analyzed the time-dependent regulation of transcription and metabolism in the gut. In the same knock-out mice, we performed a gastrointestinal infection with Candida albicans at different time of the day-night cycle, in which we assessed degree of colonization, level of inflammation, tissue damage, and immune response.

RESULTS

We demostrated that a circadian cross-talk between the three arms of trp catabolic pathway occurs in the gut. Changes in circadian transcription occurs in Ido1, Tph1 and AhR knock-out mice, which influence immune homeostasis. Moreover, the circadian regulation of trp metabolism participates to generate a time-dependent host immune response to Candida infection in the gut.

CONCLUSIONS

We demonstrated a circadian metabolic and immune modulation during gastrointestinal opportunistic infection with Candida albicans. A time-dependent partitioning of trp between host, pathogens and the microbiota might define host susceptibility to opportunistic infections at specific time of the day.

P162

THE CEREBELLUM DEVELOPMENT REGULATOR RENKCDT11 AS A NEW GENE INVOLVED IN AUTISM SPECTRUM DISORDER

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BACKGROUND-AIM

Autism spectrum disorder (ASD) is a neurodevelopmental disease characterized by restricted and repetitive behaviors, deficits in social communication, and motor difficulties. Various factors contribute to ASD etiopathology, mainly causing brain dysfunctions. However, morphological and functional abnormalities of cerebellum have been recently found in ASD, although their role in autism pathogenesis remains elusive. Our research group discovered a novel gene REN KCTD11 (REN) as a key regulator of cerebellum differentiation. Interestingly, REN belongs to KCTD family, whose members are associated with neurodevelopmental disorders, including autism.

METHODS

We performed behavior studies to evaluate reciprocal social interactions and motor functions in a knock-out REN mouse model generated in our lab (REN-KO). Cerebella morphology from REN-KO vs wild-type counterpart (REN-WT) was analyzed by Nissl-stainings. Moreover, the expression levels of autism risk genes have been examined by RT-qPCR in both brains and cerebella from REN-KO vs REN-WT mice. RNASeq analysis has been performed to identify the genes and the specific pathways deregulated in cerebella of REN-KO compared to REN-WT mice.

RESULTS

We found that the loss of REN in REN-KO mouse model determines a deficit in the behavioral profile compared to REN-WT mice showing an autistic-like behavior. This aspect correlated with morphological alterations of REN-KO cerebella, which appear bigger than their wild-type counterpart. Moreover, the molecular analysis of REN-KO cerebella vs REN-WT revealed a strong reduction of autism risk genes expression that has not been found in the rest of REN-KO brain. These results support a primary role of cerebellum in the observed deficits of this mouse model. Accordingly, RNASeq analysis of REN-KO vs REN-WT cerebella highlighted an alteration of fundamental pathways involved in neural plasticity, calcium/potassium voltage channel activity, and synaptic neurotransmission.

CONCLUSIONS

Our findings strongly suggest that the loss of REN causes structural and functional alterations of the cerebellum, impacting neural plasticity and neurotransmission, thus predisposing to ASD.

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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 defense, and for assessing the response to immunotherapeutic treatments
Within the tumor microenvironment, we have focused our attention on innate lymphoid cells (ILC) and Tγδ lymphocytes, 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 for patients.

METHODS

On a heterogeneous sample of 8 patients, we evaluated, through flow cytometry in peripheral blood in healthy mucosa and tumor-associated mucosa, the frequency of ILC subpopulations and Tγδ lymphocytes.

RESULTS

The results of the immuno-phenotypic analysis show that in samples of healthy mucosa, the frequency of ILC1 is dominant compared to that of ILC2 and ILC3; in tumor-associated mucosa samples, the frequency of ILC3 increases; in peripheral blood samples, the frequency of all three subpopulations is distributed homogeneously. Regarding Tγδ lymphocytes, in samples of healthy mucosa, their frequency appears to be higher compared to that observed in both tumor-associated mucosa and peripheral blood

CONCLUSIONS

Flow cytometry analysis of purified cell populations has provided a detailed view of phenotypic characteristics, allowing for an in-depth evaluation of surface marker expression. Characterizing cell populations is crucial for understanding the complexity of the immune response to tumors and identifying any phenotypic variations associated with the pathology. The future development of this study involves the use of functional assays to evaluate proliferative, cytotoxic, and cytokine production potential to better understand the immune dynamics in CRC and its clinical implications.

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REGULATION OF MYELOID DIFFERENTIATION THROUGH MIR-223 BINDING TO FLOTILLIN-1 PROMOTER IS SUPPRESSED IN PROMYELOCYTIC LEUKEMIA

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BACKGROUND-AIM

Mir-223 expression increases during myeloid differentiation induced by Retinoic Acid (RA). miR-223 and other miRNAs have a nuclear fraction which binds complementary DNA inducing epigenetic modifications that silence or activate gene promoters. The nuclear activity of miRNAs regulates cell lineage specification and differentiation; thus, its alteration might induce neoplastic transformation. We investigated novel functions of miR-223 searching for transcriptional targets of nuclear miR-223 during RA-induced myeloid differentiation

METHODS

Genome-wide DNA sequences bound by miR-223 were identified by ChIP-seq on Mimic miR-223-Cy5 transfected HL60 cells treated with RA. mRNAs, miRNAs and protein levels were measured by ddPCR, RT-PCR and Immunoblotting. Functional studies were carried out in myeloid cell lines by genes ectopic expression/silencing. Cell phenotype studies were used to investigate cell biology.

RESULTS

In myeloid cells undergoing RA-induced differentiation we determined by genome-wide ChIP-Seq the sequences bound by nuclear miR-223. miR-223 binds Flotillin-1 gene (FLOT1), an essential component of lipid rafts, on complementary sequences in the gene promoter. In this region miR-223 drives a complex comprising AGO1 and the Trithorax protein RBBP5 enriching H3K4me3. In hematopoietic cell lines including HL60 and NB4 and their RA-resistant mutants, Flotillin-1 RNA and protein expression depends on active RA signaling. In CD34+ hematopoietic progenitors FLOT1 expression is induced during myeloid differentiation. Its overexpression in increases vitamin D and RA-induced differentiation, whereas its knockdown produces the opposite effects. FLOT1 mRNA expression is lower in AML blasts compared to healthy bone marrow cells, the lowest expression being in APLs. mRNA and proteomics data in TGCA and MILE datasets show that FLOT1 and miR-223 have similar expression pattern in AMLs, the lowest expression levels being in APL. Only in APL patients samples RA treatment in vitro increased both FLOT1 and miR-223 expression.

CONCLUSIONS

Nuclear miR-223 transcriptionally induces the expression of FLOT1. Among them, FLOT1 enhancing myeloid differentiation. Our observations suggest an oncogenic role of aberrant expression of miR-223 and FLOT1 in AML and APL.

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CYSTIC FIBROSIS HYPERINFLAMMATION MODULATION BY SMART DRUG DELIVERY SYSTEMS FOR PROSTACYCLIN ANALOGUE REPURPOSING

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BACKGROUND-AIM

Cystic fibrosis (CF) lung disease hallmarks are the insurgence of opportunistic bacterial infections (e.g. by Pseudomonas aeruginosa), and an unresolvable inflammatory response, characterized by heightened secretion of proinflammatory cytokines. CF hyperinflammation remains an orphan drug condition. Our aim was to test smart drug-delivery systems harboring iloprost (Ilo), a synthetic prostacyclin having anti-inflammatory properties, and already approved for the treatment of pulmonary arterial hypertension, but never employed in CF

METHODS

Nasal brushings from CF patients were used to obtain airway epithelial cells, grown first through conditional reprogramming (cr) and then at Air Liquid Interface (ALI) culture conditions. Transepithelial electrical resistance (TEER) was measured by a voltohmmeter. ALI cultures were challenged with P. aeruginosa lipopolysaccharide (LPS) in the absence or presence of Nano-into-Micro (NiM) formulations (pegylated or not) containing Ilo and that were previously tested in immortalized CF bronchial epithelial cells, CFBE (doi: 10.1021/acsanm.4c01379). TNF-α, IL-6, IL-1ß, and IL-8 were analysed by real-time PCR.

RESULTS

Nasal epithelial brushings from 5 CF individuals (homozygous or compound heterozygous for the F508del mutation) were collected and isolated cells were expanded under the crc method. The increase in TEER during ALI conditions (from the 6th to the 21st day) testified the generation of a tight epithelium. IL-6 and TNF-α were significantly decreased by either free Ilo and both NIM formulations as compared with LPS only, whereas IL-1ß and IL-8 showed a trend in decrease. Since the results in CFBE cells denoted a significant downregulation for all cytokines, these results show the importance of testing primary cells from CF patients when novel drug delivery systems are evaluated.

CONCLUSIONS

The CF hyperinflammation state could be modulated by mucopenetrating nanotools in an advanced human respiratory epithelial cell model, an ex-vivo pre-clinical model for precision medicine in CF.

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B7H3 CAR-T-DERIVED EXTRACELLULAR VESICLES DISPLAY ANTITUMOR ACTIVITY IN PANCREATIC CANCER CELLS

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BACKGROUND-AIM

Pancreatic cancer (PC) has a poor prognosis and displays resistance to immunotherapy. A new frontier of cell-based immunotherapy in hematological malignancies is represented by chimeric antigen receptor (CAR)-T cells. Unfortunately, their efficacy in solid tumors is still limited. Extracellular vesicles (EVs) derived from CAR-T might circumvent these limitations. In this study, we explored the antitumor activity of CAR-T-derived EVs targeting B7H3, which is overexpressed in PC cells and resulted safe in clinical trials.

METHODS

To evaluate the cytotoxicity of engineered T cells B7H3 CAR-T and control T cells (CTRL) were sorted and co-coltured with L3.6pl positive B7H3 PC cell line. EVs from B7H3 CAR-T and CTRL-T cells were isolated and phenotypically characterized by a flow cytometry method patented by our laboratory. EVs were characterized by atomic force microscopy (AFM), western blot (WB) and nanoparticle tracking analysis (NTA). The antitumor activity of B7H3 CAR-T-derived EVs was evaluated by both MTT and 7-AAD killing assays.

RESULTS

B7H3 CAR-T-derived EVs characterization revealed that EVs displayed a globular shape and an average size of 140 nanometers. Furthermore, B7H3 CAR T-derived EVs expressed EV markers (CD63 and Flotillin-1) and were negative for Cytochrome C. Notably, B7H3 CAR-T-derived EVs showed a time-dependent killing activity against L3.6pl cells, which reflected killing effects obtained by parental B7H3 CAR-T cells in co-culture experiments with L3.6pl PC cells. In particular, with 150 μg B7H3 CAR-T-derived EVs the percentage of L3.6pl cells killing by 7-AAD was 10% ($p=0.05$) and 22% (*$p<0.05$) after 24 and 48 h treatments, respectively. In line with 7-AAD killing assay results, B7H3 CAR-T-derived EVs significantly affected PC cell viability also by MTT, as compared to control.

CONCLUSIONS

Our results showed a relevant antitumor activity of B7H3 CAR-T-derived EVs on L3.6pl PC cells in vitro, encouraging further in vitro and in vivo studies to evaluate their potential use in PC therapy.

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INTRA-PANCREATIC ADIPOSE TISSUE, INFLAMMATION AND ISLET CELL MORPHOMETRY IN OVERWEIGHT/OBESE NON-DIABETIC AND TYPE 2 DIABETIC SUBJECTS.

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BACKGROUND-AIM

Little information is available on adipose tissue features in the human pancreas (AdTP) and on their relationship with endocrine tissue characteristics and macrophage infiltration, which might play a role in metabolic dysfunctions. These features were investigated on pancreas sections from overweight/obese non-diabetic and type 2 diabetic subjects.

METHODS

Pancreas sections were obtained from 9 non-diabetic (ND, age: 65±5 yrs; 4M/5F; BMI: 31.4±0.4 Kg/m2, mean±SEM) and 13 matched type 2 diabetic (T2D, age: 71±2 yrs, 8M/5F; BMI: 30.0±0.8 kg/m2) organ donors. Adipocytes were identified and quantified, and AdTP area was assessed. Insulin (Invitrogen Ab) and glucagon (Dako Ab) positive cells were identified by immunofluorescence. Macrophages were identified by immunohistochemistry using anti-CD68 (Dako) antibody. Morphological and morphometric analyses were performed using the DM5500 Leica microscope equipped with the MetaMorph v1.8.0 software.

RESULTS

The number of adipocytes trended higher in T2D compared to ND, while their size was greater in T2D (8,371±931 μm2) than ND (5,389±608 μm2, $p<0.05$), as well as AdTP area that was higher ($p<0.05$) in T2D (6.6±0.98%) compared to ND (2.6±1.0%). Adipocytes with adjacent CD68+ cells trended higher in T2D (22.4±4.5%) compared to ND (18.1±3.5%). Insulin positive area (T2D: 0.49±0.06%; ND: 0.55±0.09%) as well as glucagon positive area (T2D: 0.24±0.03%; ND: 0.32±0.07%) did not differ significantly between the two groups. Correlation analysis showed that adipocyte size was positively associated with insulin area in ND ($p=0.019$, $r=0.75$) and negatively associated with the number of islets in T2D ($p=0.011$, $r=-0.68$). In addition, a positive relationship was observed between the number of adipocytes and the glucagon area in T2D ($p=0.019$, $r=0.64$).

CONCLUSIONS

Differences in the AdTP of T2D as compared to ND, and their correlation with islet features, suggest that in the two populations adipocytes might have different biological characteristics with possible effects on the endocrine tissue.

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TARGETING A549 LUNG CANCER CELLS: NANOURCHINS INDUCE OXIDATIVE STRESS AND AUTOPHAGY THROUGH MITOCHONDRIAL DAMAGE

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BACKGROUND-AIM

Lung cancer represents one of the most common malignant tumors, associated with high morbidity and mortality, especially for the non-small-cell lung cancer (NSCLC) which constitutes approximately 85% of all lung cancers. In the treatment of advanced NSCLC, surgical resection, chemotherapy, and radiotherapy stand as the cornerstone modalities, with platinum-based chemotherapy, like cisplatin (CDDP), commonly serving as first-line therapy in this regimen. Although some benefits of chemotherapy, this kind of treatment is very toxic to normal tissues, and it often correlates with intrinsic or acquired resistance of tumor cells. For these reasons new therapeutical approaches are required to avoid tumor relapse and progression. Nanotechnology is an exponentially growing field in oncology due to the unique physical and chemical properties of nanoparticles (NPs), which can be harnessed to selectively target and influence tumor cells. Among NPs, nanourchins, showed a great ability to enter cells thanks to their unique shape, surface features and plasmonic properties, so they should be useful for tumor treatment.

METHODS

$AuFe_3O_4$ nanourchins were tested on A549 lung cancer cells and viability was measured through MTT assay. The effects of nanourchins on A549 cells were further examined by analyzing levels of DNA strand breaks and ROS production were assessed through Western blotting analysis and BODIPY staining, respectively. The impact on cellular invasiveness was measured on Matrigel-coated polycarbonate filters with 8 #m pore size. Mitochondrial functionality after nanourchins treatment was assessed using the Seahorse XF96 Extracellular Flux Analyzer through Seahorse XF Mito Stress Test Kit.

RESULTS

Treatment with nanourchins led to increased levels of reactive oxygen species (ROS) and cellular damage, along with a diminished cell invasiveness. When tested using Seahorse XF96 Extracellular Flux Analyzer, nanourchins decreased mitochondrial functionality measured by ATP production.

CONCLUSIONS

These findings indicate that the internalization of nanourchins by A549 cells induces cellular damage and attenuates cell aggressiveness. Consequently, nanourchins hold promise as agents capable of inducing oxidative stress in A549 cells, thereby impeding tumor aggressiveness and inhibiting tumor progression.

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IN SILICO PREDICTION OF BRCA1 AND BRCA2 VARIANTS WITH CONFLICTING CLINICAL INTERPRETATION IN A COHORT OF BREAST CANCER PATIENTS

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BACKGROUND-AIM

Germline BRCA1/2 alteration have been linked to an increased risk of hereditary breast and ovarian cancer syndromes. As a result, genetic testing, based on NGS, allows to identify a high number of Variants of Uncertain Significance (VUS) or Conflicting Interpretation of Pathogenicity (CIP) variants. The identification of CIP/VUS is often considered as inconclusive and clinically not actionable for the patients' and unaffected carriers' management. In this context, their assessment and classification remain a significant challenge. The aim of the study was to investigate whether the in silico prediction tools (PolyPhen-2, SIFT, Mutation Taster, and PROVEAN) could predict the potential clinical impact and significance of BRCA1/2 CIP/VUS alterations, eventually impacting the clinical management of Breast Cancer (BC) subjects.

METHODS

We conducted a study on BRCA1 and BRCA2 CIP/VUS in our cohort of BC patients using PolyPhen-2, SIFT, MT, and PROVEAN in silico tools.

RESULTS

In a cohort of 860 BC patients, 10.6% harbored BRCA1 or BRCA2 CIP/VUS alterations, mostly observed in BRCA2 sequence (85%). Among them, 42/55 alterations were predicted as damaging at least with one in silico used tools. Prediction agreement of the 4 tools was achieved in 45.5%. Moreover, a highest consensus was obtained in 12/42 (28.6%) mutations by considering 3 out 4 in silico algorithms.

CONCLUSIONS

The use of prediction tools may help to identify variants with a potentially damaging effect. However, the lack of substantial agreement between the different algorithms suggests that the bioinformatic approaches should be combined with the personal and family history of the cancer patients.

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ALTERED POSTTRANSCRIPTIONAL GENE REGULATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: ROLE OF THE RNA-BINDING PROTEIN AUF-1 IN AIRWAY EPITHELIAL RESPONSES

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BACKGROUND-AIM

Chronic obstructive pulmonary disease (COPD) is defined by persistent lung inflammation elicited by exposure to environmental noxious stimuli (ENS), chiefly cigarette smoke (CS), leading to airway remodeling, destruction of lung parenchyma, emphysema and impaired lung function. ENS/CS increase respiratory oxidative stress (OS) burden leading, in genetically at risk subjects with defective antioxidant response, to pathogenic lung inflammation, accelerated cell senescence and senescence-associated secretory phenotype (SASP) featuring overexpression of cytokines, chemokines and other mediators. RNA-binding proteins (RBPs) are key posttranscriptional regulators of OS-driven responses, controlling mRNA processing, transport and cytoplasmic fate by forming multi-component ribonucleoprotein complexes. We previously identified the selective loss of the RBP AU-binding Factor (AUF1) in bronchiolar epithelium of COPD patients vs controls and in the human airway epithelial cell line BEAS-2B. AUF1 silencing increased epithelial senescence and SASP. AUF1 mRNA targets were enriched in COPD epithelial transcriptome. We report in this study the initial analysis of AUF1 protein interactome in BEAS-2B cells.

METHODS

Immunoprecipitation (IP) of total protein extracts from resting BEAS-2B was performed with anti-AUF1 Ab, matched Ig isotype and no Ab controls. Western blot (WB) confirmed AUF1 IP specificity. Proteins were digested, analyzed by nano LC-MS/MS, then characterized and quantified by bioinformatic analysis using Proteome Discoverer (Thermo-Fisher) and Mascot (Matrix Science) software.

RESULTS

WB showed strong protein enrichment in IP-AUF1 vs control IPs. AUF1 IP/No-Ab IP peptides were subjected to LC-MS/MS analysis. Proteins were identified by matching experimental data with Uniprot KB/Swiss-prot human protein database. Putative AUF1 interactors were selected among proteins displaying a fold change $\geq$ 1.5 between AUF1 IP vs No-Ab IP samples. Myosin-9, Myosin light polypeptide 6, Actin and Fatty acid-binding protein 5 were the highest-scored proteins. In parallel, genome ontology database analysis showed AUF1-bound mRNAs expressed in COPD lung samples as significantly enriched in RNA-Seq transcriptome regulated by the RBP LIN28, suggesting a functional/spatial relationship between AUF1/LIN28.

CONCLUSIONS

This initial identification of putative AUF1-associated factors is part of the ongoing study of altered posttranscriptional regulatory mechanisms in COPD pathogenesis.

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MOLECULAR DIAGNOSIS OF VON WILLEBRAND DISEASE BY NEXT GENERATION SEQUENCING: A CASE REPORT

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BACKGROUND-AIM

Von Willebrand disease (VWD) is a congenital haemorrhagic disorder characterised by partial or total quantitative (type 1 and 3 disease) or qualitative (type 2A, 2B, 2M and 2N disease) deficiency of von Willebrand factor (VWF).
Diagnosis and classification of VWD can be performed by first-level tests, like quantitative determination of von Willebrand factor antigen (VWF:Ag) and VWF ristocetin cofactor activity (VWF:Rco), second-level tests, which include determination of VWF collagen-binding activity (VWF:CB), and third-level tests, like analysis of the multimeric distribution of VWF by electrophoresis and immunofixation. Prospects for diagnosis of VWD have changed dramatically in recent years with the unveiling of Next Generation Sequencing (NGS) platforms. NGS is crucial in confirming diagnosis and classification of the type/subtype of VWD, and could guide the selection of the most appropriate therapy.

METHODS

We present a case report that illustrates the diagnostic approach when VWD is suspected. The clinical case involves an 80-year-old male patient, who complained of recurrent post-traumatic hematomas and bruises. We performed VWF:Ag, VWF:Rco and VWF:CB determinations, multimeric distribution analysis of VWF and genetic analysis of VWF by NGS.

RESULTS

The quantitative determination of VWF:Ag (2%), VWF:Rco (1%) and VWF:CB (<0.50%) led to the clinical suspicion of type 3 VWD, characterised by an almost complete loss of VWF. Analysis of the multimeric distribution of VWF further confirmed the absence of VWF: none of the VWF multimers could be detected by immunofixation. These findings allowed the presumptive diagnosis of VWD type 3. To confirm diagnosis and to identify the mutation responsible for VWD, NGS was performed and showed the presence of GRCh38_12:6046734, exon 16, frameshift variant, c.2269_2270del-p.Leu757ValfsTer22, in homozygosity. A definitive diagnosis of VWD type 3 was made and an appropriate therapy was selected.

CONCLUSIONS

Genetic studies based on the use of NGS to perform genomic characterization of patients have strong translational power, as they can contribute to the identification of new factors that aggravate the clinical picture of patients and to the development of increasingly specific and targeted therapies.

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MESOPOROUS SILICA-BASED NANOSYSTEMS FOR THE TARGETED DELIVERY OF DOXORUBICIN

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BACKGROUND-AIM

Cancer represents a major burden worldwide, responsible for almost 20% deaths in 2021. One of the main challenges in cancer treatment is to develop a therapeutic strategy able to selectively target tumor cells preserving normal tissues from unwanted side effects. Localized drug delivery should cope this aim. A mesoporous silica-based nanodevice, bearing the chemotherapy drug doxorubicin (DOXO), whose release is triggered by the acidic tumor environment, and the targeting function folic acid (FOL), was developed (FOL-MSN-DOXO) and tested in vitro against folate receptor expressing (FR+) cancer cells as well as on FR negative (FR-) normal (healthy) cells.

METHODS

FOL-MSN-DOXO efficacy studies were conducted by means of growth experiments, TEM, TUNEL, Annexin and ROS assays.

RESULTS

FOL-MSN-DOXO is able to kill FR+ cancer cells, but not FR- normal cells, while free DOXO resulted toxic for all cell lines tested, regardless of FR expression. MSNs uptake occurred exclusively in FR+ cells, through FR-mediated endocytosis, while no uptake was observed in FR- cells. Both FOL-MSN-DOXO and free DOXO led to a significant increase in ROS production and, consequently, in the apoptotic rate of FR+ cells, but only the free drug was able to induce ROS and, in turn, to trigger apoptosis in FR- normal cells. Notably, the vehicle alone (FOL-MSN) was not toxic in any of the cell models tested.

CONCLUSIONS

Due to its specific targeting towards FR-expressing cancer cells, the FOL-MSN-DOXO nanosystem demonstrates improved safety and significantly increased antitumor efficacy compared to conventional doxorubicin formulations. As a result, it represents a promising strategy for the targeted and safe delivery of the chemotherapy drug in the treatments of FR expressing cancers.

P173

EXPLORING THE ROLE OF MICRORNAS IN INTERCELLULAR COMMUNICATION DURING IMMUNOGENIC CELL DEATH

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BACKGROUND-AIM

Immunogenic Cell Death (ICD) is a therapy-induced phenomenon where dying tumor cells expose and release numerous damage-associated molecular patterns (DAMPs) that trigger an immune response especially interacting with dendritic cells (DCs). Recently, we found significant alterations in microRNA profile in lymphoma cells (Mino) after ICD induction by treatment with the combination of retinoic acid (RA) and interferon-α (IFNα). RA/IFNα-treatment markedly upregulated miR-212-3p and miR-4284, among others, both involved in immune processes. Tumor cell lysates obtained from RA/IFNα-treated Mino cells (RA/IFNα-TCLs) proved effective reservoirs of tumor antigens and adjuvants for loading monocyte-derived DCs. Indeed, RA/IFNα-TCLs-pulsed DCs were more efficient in eliciting specific antitumor T cell response compared to CTRL-TCL-pulsed DCs. Herein, we aim to unravel the contribution of ICD-induced microRNAs in the interaction between dying tumor cells and DCs.

METHODS

RA/IFNα-TCLs/CTRL-TCLs from treated/untreated lymphoma cell line Mino were used for loading monocyte-derived DCs from healthy donors. Extracellular vesicles (EVs) were isolated by ultracentrifugation from untreated/treated Mino cells and characterized by nanoparticle analyzer. MicroRNAs were evaluated by next-generation sequencing (NGS) and real-time PCR; secreted cytokines through multiplex beads-based assay.

RESULTS

NGS analyses revealed upregulation of both miR-212-3p and miR-4284 in RA/IFNα-TCLs and in RA/IFNα-TCL-pulsed DCs compared to CTRL-TCL-pulsed DCs, with miR-212-3p confirmed by real-time PCR. Functional network analysis unveiled interactions between miR-212-3p and predicted mRNA targets, notably within cell surface receptor signaling pathways, like the IL-1 pathway. This finding was further supported by cytokine secretion patterns. We hypothesized the contribution of microRNAs to tumor-DCs cross-talk during in situ ICD, thus we explored their presence in tumor extracellular vesicles (EVs) as mediators of intercellular communication. The analysis of EVs isolated from the culture medium of RA/IFNα-treated Mino cells showed an increase in concentration and size respect to EVs released by untreated cells. Notably, real-time PCR showed significant increase of miR-212-3p levels in EVs derived from RA/IFNα-treated tumor cells.

CONCLUSIONS

Our results described the involvement of microRNAs and their transport in EVs in orchestrating immune mechanisms during experimental ICD with potential implications for clinical applications.

P174

EVALUATION OF THE EFFECTIVENESS OF S. THERMOPHILUS LYSATE IN COUNTERACTING THE BIOMOLECULAR EVENTS ASSOCIATED WITH AGING OF HUMAN DERMAL FIBROBLASTS

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BACKGROUND-AIM

Skin aging is a progressive process characterized by many structural and physiological changes in the skin. Human dermal fibroblasts (HDF) act as a crucial player in the skin homeostasis. When exposed to environmental and oxidative stress, they develop a senescent phenotype, contributing to functional defects in the skin related to aging. Growing evidence supports the use of topical probiotics in treating various skin conditions, including skin aging. The anti-aging potential of Streptococcus thermophilus, reported in vitro and in vivo, has encouraged us to focus on the biomolecular mechanisms underlying its effects, evaluating the reparative ability of S. thermophilus lysate in an H2O2-induced human dermal fibroblast senescence model. The effects of different concentrations of probiotic lysate on oxidative stress and inflammatory profile, both related to aging, were investigated.

METHODS

An oxidative stress-induced senescence model was established by H2O2 exposure on HDF. The effects of probiotic lysate at different concentrations were studied on proliferation, evaluated by the IncuCyte® system, on collagen I synthesis, nuclear factor E2-related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB), assessed by western blotting. Moreover, the oxidative stress markers (ROS, malondialdehyde -MDA), catalase and superoxide dismutase levels, and IL-1ß and IL-6 concentration were assayed by commercial kits.

RESULTS

S. thermophilus lysate exposure increased the proliferation, counteracted the H2O2-induced senescence by reducing aging-associated markers, and promoting collagen I synthesis. Of note, S. thermophilus lysate lowered cellular aging-associated oxidative damage by effectively decreasing ROS and MDA levels, as well as increasing antioxidant enzyme activities through the activation of the Nrf2 in aged HDFs and inhibition of the NF-κB pathway, inducing a downregulation of pro-inflammatory markers.

CONCLUSIONS

Overall, our results evidence that S. thermophilus lysate, by inhibiting NF-κB expression and restoring Nrf2 phosphorylation, was able to re-establish the physiologic functions of aged HDFs, including cell proliferation, collagen synthesis, and antioxidant systems, thus supporting the reparative action of this probiotic in treating skin aging.

P175

EXPLORING THE IMPACT OF NANOPLASTICS ON THE BONE MICROENVIRONMENT: THE ROLE OF EXTRACELLULAR VESICLE-MEDIATED COMMUNICATION AND OXIDATIVE STRESS IN MULTIPLE MYELOMA.

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BACKGROUND-AIM

Nanoplastics (NPs) are a novel, worrisome issue in environmental and human well-being studies due to their capability to cross cell membranes and induce cellular toxicity. Recent studies have shown that the toxic effects of NPs are mediated by oxidative stress (OS), a critical player in disrupting skeletal integrity. Consequently, once NPs infiltrate the bone, they exert detrimental effects under normal and pathological conditions, such as multiple myeloma (MM).
Bone represents the elective tumoral niche for MM, which alters the local microenvironment, impairing osteoblastogenesis. Tumor-derived extracellular vesicles (EVs) play a crucial role In creating a supportive bone marrow (BM) environment for MM by transferring specific molecular signals in their cargo. Among these, miR505 regulates the osteoblastogenic factor Runx2.
Here, we explored the effect of NPs in MM by investigating if NPs represent a source OS for MM cells, affecting their viability and evaluating their impact on EV-mediated communication.

METHODS

OPM2, HS5, and MC3T-E1 were used for the in vitro studies. Cells were exposed to NPs (1-200 ug/ml) for 48 hours. Cell viability and proliferation were determined by MTT assay. ROS level was determined by DCF assay. mRNA and miRNA levels were analyzed by q-PCR. The profile of the EVs was determined by NTA and spectral flow cytometer.

RESULTS

We found that NPs uptaken by stromal and MM cells reduce cell viability and proliferation due to ROS production. This is consistent with the induction of oxidative stress response genes such as heme oxygenase 1, an inducible gene controlled by the Nrf2/ARE pathway. Moreover, NPs affect the communication between MM cells and the BM microenvironment mediated by EVs. Indeed, MM-derived EVs show reduced diameter and higher concentration after NP exposure. Also, NP exposure disrupts MM-EVs' ability to create a supportive microenvironment by inhibiting osteogenesis, as assessed by Runx2 gene expression analysis. The underlying molecular mechanism involves a decrease in vesicular miR505 levels.

CONCLUSIONS

This work unravels the potential therapeutic role of NPs in MM, suggesting that they may exert a cytotoxic effect on MM cells and hamper the malicious EV-mediated crosstalk between MM cells and the BM microenvironment, resulting in bone disease.

P176

A CIRCADIAN CLOCK IN SERTOLI CELLS PROTECTS TESTIS FROM GONADOTOXIC DAMAGE

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BACKGROUND-AIM

Chemotherapy has serious consequences for testicular function in male patients. While adults can cryopreserve seminal fluid, for prepubertal youths, still unable to produce sperm, fertility restoration strategies are still experimental and include cryopreservation of testicular tissue as well as pharmacological approaches to protect testicular cells from gonadotoxic damage. In the last years, many studies have demonstrated that the sensitivity of cells to genotoxic stress relies, among others, on the presence of a functional circadian clock, controlling many aspects of cell physiology. In the testis, daily rhythms of endocrine male function have been widely reported in mammals. Our project aims at evaluating the protective capacity of pharmacological modulators of circadian rhythms on testicular cells exposed to anticancer treatment, by focusing on Sertoli cells, which sustain spermatogenesis through fundamental endocrine, metabolic and immunological functions.

METHODS

We performed in vitro studies in TM4 murine Sertoli cell line and in porcine prepubertal primary Sertoli cells. The presence of a functional circadian clock in these cells was assessed by analyzing gene and protein expression at different time of the day in synchronized cells. Thereafter, evaluation of cell viability, cell cycle and DNA damage response, inflammatory response and markers of endocrine function was performed in Sertoli cells exposed to chemotherapeutic agent doxorubicin or cisplatin and treated with modulators of circadian rhythm.

RESULTS

We obtained results showing that both murine and porcine Sertoli cells have a robust circadian gene expression. In these cells, the circadian modulator nobiletin displays a cytoprotective effect against doxorubicin injury. Indeed, nobiletin counteracts the chemotherapeutic toxicity by enhancing cell viability, reducing apoptosis and modulating the expression of pro-inflammatory cytokines in a time-dependent manner.

CONCLUSIONS

Results indicates that the circadian clock is directly involved in the regulation of Sertoli cells physiology, as well as their response to anticancer treatments. Identifying circadian modulators as protective agents against chemotherapy-induced gonadotoxic damage may be an innovative pharmacological approach to preserve testicular function undermined by chemotherapy administration.

P177

DECIPHERING PVT1-MEDIATED TRANSCRIPTIONAL MODULATION IN ERα POSITIVE BREAST CANCER CELLS

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BACKGROUND-AIM

In hormone responsive breast cancer (BC), an aberrant transcription can lead to deregulation of target genes, thus involving coding and non-coding molecules closely associated with tumorigenesis and metastasis. Long-noncoding RNAs (lncRNAs) represent promising therapeutic innovations as key molecular partners for RNA binding proteins (RBPs) in the regulation of gene expression. Since the Estrogen Receptor α (ERα) acts as RBP, we investigated novel druggable molecules among essential lncRNAs interacting with the receptor in chromatin-associated multi-molecular complexes formation.

METHODS

ASO-mediated gene silencing was applied to evaluate the functional effects of the essential lncRNA PVT1 on cell proliferation, migration, apoptosis and its impact on the estrogenic signaling in both responsive and anti-estrogen resistant cell models. Then, the transcriptome of MCF-7 cell clones holding an inactivating mutation of the ERα RNA binding domain (RBD) was profiled and compared to that of PVT1-silenced MCF7 cells.

RESULTS

The RNA binding capability seems to be required in several ERα-modulated BC hallmarks suggesting the involvement of RNA molecules in the modulation of the receptor oncogenic activity. Indeed, among ERα-interacting lncRNAs, the functional screening suggested the oncogenic lncRNA PVT1 as putative candidate for BC treatment even in presence of antiestrogen resistance. Furthermore, among common deregulated genes following PVT1 silencing or in presence of mutant ERα RBD, those enrolled in estrogen signaling were retrieved. In this regulatory landscape, we hypothesized a complex engaging PVT1 as modulator of chromatin architecture and accessibility and mediating the interaction between ERα and the histone methyltransferase EZH2. This nuclear cooperating machinery was proved to regulate the expression of specific target genes.

CONCLUSIONS

Emerging at the forefront of cancer research, targeting lncRNAs offers promising avenues for diagnostic and therapeutic innovations being pivotal molecules in driving tumor growth and conferring resistance to therapy. In this study, PVT1 was selected among others because, bridging the gap between ERα and EZH2, can coordinate the expression of genes involved in breast carcinogenesis.

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P178

MICROFLUIDIC MULTI-ORGAN-ON-A-CHIP IN TRANSLATIONAL PATHOLOGY: A CASE FOR HYPEROXALURIA.

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BACKGROUND-AIM

Organ-on-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. In this study, we applied this technology to hyperoxaluria, a medical condition characterized by calcium oxalate urinary super saturation, oxalate crystals deposition, and kidney damage that can be either primary (PH) or secondary (SH) to other conditions, such as those characterized by fat malabsorption. Although it represents the most widespread form, few studies have explored the causal mechanisms of SH hampering diagnostic and therapeutic advances. Based on these premises, we developed a gut-kidney multi-OoC system to deeper characterize the altered oxalate distribution in SH.

METHODS

The OoC system was based on the HUMIMIC Chip4 technology (TissUse Gmbh, Berlin, Germany), that accommodates both an intestinal barrier (differentiated Caco2 cells grown on top of a transwell) and a kidney model made up of both glomerular and tubular cells, allowing to assess oxalate distribution over time and upon different conditions.

RESULTS

We first analyzed the intestine and kidney components separately following exposure to oxalate. On the one hand, Caco2 cells were able to transport oxalate in both the apical-to-basolateral and the basolateral-to-apical directions. Chemical (Dextran Sodium Sulfate) and mechanical (scratching) stress promoted basolateral oxalate accumulation. On the other hand, cultured proximal tubular cells and podocytes were dose-dependently damaged by oxalate. The different components were then assembled in the OoC system, and oxalate absorption in the intestinal mucosa and excretion in the urine could be reproduced in normal and perturbed conditions.

CONCLUSIONS

The development of a multi-organ-on-chip model could represent an innovative study system in translational pathology with the potential to identify pathogenic mechanisms and validate novel therapeutic strategies.

P179

A NIOSOME-BASED DELIVERY SYSTEM FOR PHENFORMIN-MEDIATED REDOX ALTERATIONS TARGETING IN MEDULLOBLASTOMA TUMORS

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BACKGROUND-AIM

The biguanide phenformin is an antidiabetic drug known for its potent anticancer properties in preclinical and clinical settings. We have recently demonstrated that in Shh Medulloblastoma (MB), micromolar doses of phenformin increase NADH/NAD+ ratio, thus promoting an antitumor response.
However, the clinical use of phenformin is currently limited due to its toxicity. In order to counteract phenformin potential side effects, we encapsulated the drug into nanocarriers able to direct its selective delivery to MB tissue. In this work, we have designed, synthesized and characterized specific phenformin-loaded nanovectors, called niosomes, and we have tested their therapeutic benefit in cultures of Mb cells and in MB animal models.

METHODS

Phenformin loaded niosomes were synthesized by film method in order to reduce carrier-induced toxicity and extend plasma halflife. Med1-MB cells were exposed to increasing amounts of niosomes to evaluate the in vitro cytotoxicity, and the viability of cells was determined using the Promega CellTiter-Glo Luminescent Cell Viability Assay. To study cellular uptake, cells were treated as above, collected, and examined under a fluorescence microscope. To investigate the in vivo pharmacokinetic distribution, mice were administered with phenformin-loaded niosomes by tail vein injections. Samples of plasma and tissue were collected two hours post-injection. Using qRT-PCR analysis on samples from both in vitro and in vivo settings, we validated the effect of the treatment on Shh signaling.

RESULTS

Our results demonstrate that the use of phenformin-loaded niosomes enhances the drug anti-proliferative effects by markedly increasing its concentration and redox state in MB cells. When administered to MB mouse models, phenformin-loaded niosomes enhanced drug concentration in the brain and strengthened the oncogenic inhibition.

CONCLUSIONS

Together, these data demonstrate that the use of phenformin-loaded niosomes strongly enhances the concentration of the drug, maximizing the biguanide-mediated redox imbalance. In addition, the administration of this niosome-based delivery system results in a higher accumulation of drug in the brain and an enhanced anticancer effect, suggesting that this novel system could be an effective new strategy against brain tumors.

P180

TARGETING EPIGENETIC VULNERABILITIES TO OVERCOME ENDOCRINE THERAPY RESISTANCE IN BREAST CANCER

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BACKGROUND-AIM

Exploiting the epigenetic vulnerabilities of cancer cells represents an effective strategy for identifying new therapeutic targets to overcome resistance to current pharmacological regimens. In breast cancer (BC), in particular, endocrine therapy (ET) resistance arises mostly from constitutive or aberrant activation of the Estrogen Receptor alpha (ERα) signaling pathway. Thus, targeting the epigenetic factors involved in this receptor-mediated signaling pathway represents a rational approach for developing new potential treatments against these deadly neoplasms.

METHODS

We combined functional genomics and bioinformatics analysis, siRNA-mediated gene knockdown, transcriptome profiling, and pharmacological inhibition, coupled with cellular and functional assays in antiestrogen (AE)-sensitive and -resistant human BC cell models, to characterize the impact of these epigenetic regulators on the progression and survival of luminal-like ERα+ BC models.

RESULTS

We uncovered a critical involvement of histone modifiers in controlling gene transcription in key BC functions. Specific epigenetic inhibitors were able to block the proliferation of AE- (tamoxifen or fulvestrant) sensitive and resistant BC cells via transcriptome modifications, which resulted, among others, in the inhibition of crucial BC pathways such as the cell cycle and epithelial-to-mesenchymal transition.

CONCLUSIONS

The identification of new epigenetic vulnerabilities involved in the estrogenic signaling cascade, which impact the proliferation and survival of ET-resistant BC cells, has revealed new potential therapeutic approaches for the management of these aggressive tumors.

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P181

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED MICRORNAS AS POTENTIAL BIOMARKERS IN GLAUCOMA: STUDY ON IN VITRO AND IN VIVO GLAUCOMA MODELS.

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BACKGROUND-AIM

Dysregulated microRNA (miRNAs) expression is observed and closely related to many human diseases, including glaucoma, a neurodegenerative disease characterized by progressive death of retinal ganglion cells and optic nerve axons, mostly caused by increased intraocular pressure. Although many risk factors have been identified, glaucoma pathogenesis is largely unknown. Here, miRNA profiling was performed in in vitro and in vivo glaucoma models to provide novel insights into the potential involvement of such molecules in glaucoma pathogenesis.

METHODS

In vitro analysis was performed using a LiveFlow millifluidic device (IVTech) able to mimic the glaucomatous condition, where rat müller cells (rMC-1) were placed under basal flow (CTR) and increased hydrostatic pressure (HP). In vivo study was carried out on retinas from the glaucoma predisposed DBA/2J mice. MiRNAs expression levels were analyzed by TaqMan-based RT-qPCR, comparing glaucomatous vs. non-glaucomatous conditions. Significant differentially expressed miRNAs were examined in silico by DIANA-miRpath and Tarbase to identify target genes and pathways, and further analyzed at the functional level.

RESULTS

MiRNA profiling revealed 28 and 11 significantly dysregulated miRNAs in HP rMC-1 and glaucomatous mice retinas, respectively. MiRNAs-related significant pathways, involved in biological processes already described in retina pathophysiology and implicated in glaucoma, were identified. The oxidative and inflammatory mechanisms characteristic of glaucoma were investigated through an analysis of the identified target genes, including Il6, TNFα, Nrf2, Dj1, and Cat.
MiR-652-3p, miR-28-5p and miR-301a-3p were common to both glaucoma models, and functional activity was confirmed by in vitro assay, providing a preliminary validation of in silico data.

CONCLUSIONS

Novel differentially expressed miRNAs, involved in key signalling pathways, were identified in in vitro and in vivo glaucoma models. Among them, three were common to both models, suggesting a more specific involvement in the pathogenesis of the disease and a putative role as biomarkers or therapeutic targets, thus opening to further validation analyses.

P182

ANTIFIBROTIC TARGETING CANCER-ASSOCIATED FIBROBLASTS ALTERS TUMOR MICROENVIRONMENT, REDUCING NSCLC PROLIFERATION VIA NRF2 MODULATION

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BACKGROUND-AIM

Lung cancer remains one of the most commonly diagnosed malignancies, with non-small cell lung cancer (NSCLC) being the predominant histological type. Clinical and preclinical studies suggest that NSCLC aggressiveness relys on the complex interactions within the tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) are crucial components of the TME, fostering a fibrotic environment that protects cancer cells and hinders therapeutic efficacy. Pirfenidone (PFD), an antifibrotic drug targets TGFβ signaling, inhibits CAFs differentiation and reduces extracellular matrix (ECM) proteins deposition.In this preclinical study we explored the effects of a PFD-altered TME on NSCLC cell aggressiveness evaluating epithelial-mesenchymal transition (EMT) and stemness markers, redox state, proliferation and viability, to highlight potential therapeutic targets within tumor-stroma interactions.

METHODS

CAFs, isolated from human NSCLC patients, were exposed to PFD (1.5 mg/ml). NSCLC cell lines, A549, H1975 and H1299 were treated with CAFs-conditioned medium (CM-CAF and CM-CAF-PFD) for 24 hours, and analysed using real-time PCR, western blot, immunofluorescence and flow cytometry.

RESULTS

The exposure of NSCLC cells to PFD-CAF-CM influences multiple cellular processes, specifically: i)We found that cell viability and proliferation were reduced in cancer cell exposed to PFD-CAF-CM compared to CAF-CM, and that this reduction correlates with the modulation of Cyclin D1 and p21 involved in cell cycle regulation, with a partial increase in the cell population that undergoes death; ii)NSCLC cells exposed to PFD-CAF-CM exhibited Nrf2 transcription factor downregulation and a decrease in its nuclear translocation, with an increase in the production of reactive oxygen species (ROS);iii) NSCLC cells exposed to PFD-CAF-CM showed a reduced clonogenic ability.

CONCLUSIONS

We suggest that PFD exerts a complementary unpredicted anticancer effect targeting Nrf2 pathway perturbating cancer cells-CAFs crosstalk. These findings highlight Nrf2 as a potential therapeutic target. We Thus, Nrf2 will be used as a target for anticancer treatment based on RNA technology in accordance with $NextgenerationUE PNRR 2022 - CN 3 - National Center for Gene Therapy and Drugs based on RNA Technology - Spoke 2 - PhD program: The cross-talk between stroma and cancer cells in the tumor microenvironment as a target in therapies customized RNA.

P183

THE BENEFICIAL ANTI-INFLAMMATORY AND ANTI-OXIDANT EFFECTS OF SICILIAN EXTRA VIRGIN OLIVE OIL POLYPHENOLS-ENRICHED EXTRACTS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Rheumatoid arthritis (RA) is a T-cell-mediated chronic systemic autoimmune disease. Recent findings emphasize the anti-inflammatory and antioxidant properties of bioactive natural compounds, such as polyphenols, and their possible use in synergy with current therapies to improve the prognosis and symptoms of RA. Experimental evidence demonstrated the beneficial effects of Extra Virgin Olive Oil polyphenols-enriched extracts (PE-EVOOs) on the reduction of intracellular pro-inflammatory cytokines, such as IL-1β and TNF-α and ROS in the PBMCs of RA patients.

METHODS

EVOO is dissolved in hexane and a mixture of EtOH/H2O (8:2), vortexed, and centrifuged. The polar organic phases obtained were combined, and the content of Gallic Acid equivalents was detected using the Folin-Ciocalteu protocol. The radical scavenging activity of PE-EVOOs was determined by DPPH assay. PBMCs isolated from patients with RA and healthy subjects were treated with 75μg/ml of PE-EVOOs for 48h alone and for another 4h with 5μg/ml of LPS, respectively, to evaluate their ability to reduce the intracellular production of pro-inflammatory cytokines (TNF-α and IL-1β) and ROS, which were quantified through the oxidation of the H2DCFDA dye. ROS and cytokines production was evaluated by Flow cytometry.

RESULTS

By MTT assays, we determined that 75μg/ml of PE-EVOOs as optimal working concentration. Findings showed that 48h pre-treatment caused a 2-fold reduction of intracellular ROS production in PBMCs from RA patients and healthy subjects treated with LPS (5μg/ml) for 4h. These findings highlighted the antioxidant power of PE-EVOOs, confirmed by DPPH radical scavenging assay, which demonstrated elevated and dose-dependent radical anti-oxidant activity (from 25% reached by 1.56μg/ml up to 98% reached by 62.5μg/ml). Finally, flow cytometry analysis of PBMCs from these subjects showed a 35-fold reduction for TNF-α and a 15-fold reduction for IL-1β intracellular production after the same pre-treatment with PE-EVOOs, thus demonstrating their strong anti-inflammatory properties.

CONCLUSIONS

Our preliminary results demonstrate the anti-inflammatory potential of PE-EVOOs, as they appear to reduce intracellular ROS and pro-inflammatory cytokine production in PBMCs from RA patients and LPS-treated healthy subjects. Further experiments will be conducted to confirm these findings and identify the specific polyphenols in the oil that most effectively contribute to its anti-inflammatory and antioxidant effects.

P184

A NEW BISPECIFIC CONSTRUCT PROMISES AN EFFECTIVE TCR-BASED ANTI-TUBERCULAR THERAPY

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BACKGROUND-AIM

Several studies have identified a subset of HLA-E-restricted CD8+ T cells that recognize peptides derived from Mycobacterium tuberculosis (Mtb). These cells produce cytokines and exhibit cytotoxic activity. HLA-E is a non-classical HLA Ib molecule with very limited polymorphism, making it an attractive target for TCR-based immunotherapies. Recently, a new class of bispecific constructs, based on HLA-E recognition and activating T cells, has shown promising results in TCR-based cancer and infectious diseases therapies. This study examines the potential of a novel TCR-based bispecific molecule (ImmTAB-inhA®) developed by Immunocore (Oxford, UK). This molecule selectively binds to HLA-E in complex with a peptide (p44) belonging to the enoyl reductase enzyme coded by the inhA gene of Mtb. This molecule can redirect T cells to target HLA-E-expressing p44 on the surface of cells infected with Mtb..

METHODS

We used RMA-S cells expressing human HLA-E pulsed with p44 to assess cytokine production. PBMC from healthy donors were cultured with these cells in the presence of ImmTAB-inhA®, and flow cytometry assessed cytokine production. To test the antimicrobial potential of ImmTAB-inhA® by luminometry, we cultured THP1-derived macrophages infected with chemoluminescent H37RV Mtb with varying concentrations of ImmTAB-inhA® and PBMC from healthy donors, with or without antibiotics.

RESULTS

In the co-culture of RMA-S and PBMC, ImmTAB-inhA® determined the polyclonal engagement of a significant percentage of T cells that produce IFN-γ and TNF-α. Additionally, ImmTAB-inhA® yields a substantial reduction in Mtb CFU compared to the control without ImmTAB-inhA ®in the THP1 co-cultures with PBMC.

CONCLUSIONS

ImmuTAB-inhA® is highly effective in redirecting the activation of T cells against target cells infected with Mtb. Hence, this construct is a promising tool for developing TCR-based immunotherapy against tuberculosis that can overcome HLA restriction and synergise with antibiotic therapy.

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NOVEL SYNTHETIC LIGANDS OF THE FORMYL PEPTIDE RECEPTORS AS POTENTIAL THERAPEUTICS FOR COLORECTAL INFLAMMATORY DISEASES

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BACKGROUND-AIM

The formyl peptide receptors (FPRs) are G protein-coupled receptors that transduce chemotactic signals in phagocytes and mediate host-defense as well as inflammatory responses. They are expressed in various cell types, including colon epithelial cells, and their activity is critical for homeostasis, inflammation, and epithelial repair processes. The human FPR family includes FPR1, FPR2/ALX, and FPR3.
This study aimed to synthesize and evaluate the potential anti-inflammatory effects of 14 novel ligand-based peptides (AMGS 1-14) targeting FPRs for the treatment of colorectal inflammatory diseases.

METHODS

The in vivo effects of the peptides were screened using a formalin test on mice, followed by an MTT cytotoxicity assay to assess their in vitro effects on Caco2 and WiDr cells at several concentrations. The effects on inflammation were evaluated by treating cells with AMGS 9 and AMGS10 at 1 μM, with or without LPS stimulation, and analyzing the expression of pro- and anti-inflammatory genes.

RESULTS

AMGS9 was the most active peptide in eliciting anti-inflammatory responses in vivo, along with AMGS3, AMGS6, AMGS10, and AMGS14. A concentration of 1 μM was found to be non-toxic to WiDr cells. The treatment exhibited anti-inflammatory effects by decreasing the expression of genes associated with inflammation, such as IL-1β, IL-6, TNF-α, the COX-2 enzyme involved in prostaglandin synthesis, and p65 and p50 subunits of the NF-KB complex involved in immune and inflammatory responses. Additionally, it increased the expression of anti-inflammatory genes, including TGF-β and IL-10.

CONCLUSIONS

Based on these results and further analysis, targeting FPRs with these new synthetic formylated peptides could be considered a new therapeutic approach for treating colorectal and intestinal inflammatory diseases.

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UNVEILING THE ROLE OF NOD2 IN INNATE IMMUNITY AGAINST LEISHMANIA INFANTUM PARASITES: IMPLICATIONS FOR IMMUNOTHERAPY

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BACKGROUND-AIM

Visceral leishmaniasis (VL), caused by Leishmania infantum, poses a significant global health challenge, especially in the Mediterranean basin. The emergence of drug-resistant strains and the severe toxicity of current treatments underscore the urgent need for alternative therapies, such as immunotherapy. This study explores the NOD2 (nucleotide-binding oligomerization domain 2) pathway, a crucial receptor involved in pathogen control and inflammation, and its role in recognizing and responding to L. infantum infection. Despite NOD2's established function as a sensor of bacterial peptidoglycan, its role in Leishmania infections is underexplored. We hypothesize that NOD2 would detect L. infantum's pathogen-associated molecular patterns (PAMPs), initiating a robust immune response. Elucidating this pathway could reveal novel therapeutic targets, advancing immunotherapeutic strategies against VL.

METHODS

We employed immortalized bone marrow-derived macrophages (BMDM), both primed with IFN-γ and untreated, infected with wild-type and red fluorescent L. infantum strains for live-cell imaging. NOD2 was inhibited with the specific inhibitor GSK717, while muramyl dipeptide (MDP) served as the canonical NOD2 ligand to enhance microbicidal responses. Cytokine quantification was done by ELISA, NO levels assessed with the Griess assay, and iNOS expression via qPCR and WB.

RESULTS

Consistent with prior findings, IFN-γ stimulation significantly reduced infected BMDMs and intracellular amastigote count. Conversely, NOD2 inhibition increased infection rates and parasite proliferation, indicating NOD2's crucial role in controlling L. infantum. NOD2 inhibition also markedly reduced NO levels, highlighting the importance of NOD2 activation for effective NO-mediated parasite control. To explore MDP's potential as an immunomodulator, we stimulated BMDMs with MDP in the presence of parasites. This significantly reduced infected cells and increased TNF-α production, essential for Th1 polarization and infection resolution. Notably, iNOS expression in MDP-stimulated BMDMs was 5-fold higher than in cells infected with parasites alone.

CONCLUSIONS

Collectively, these findings highlight NOD2's critical role in recognizing L. infantum and activating antiparasitic immune responses. A deeper understanding of NOD2 signaling, combined with the promising use of MDP as an adjuvant or immunomodulator, could pave the way for innovative immunotherapies, offering new hope against this neglected and deadly disease.

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NOVEL N-(HETEROCYCLYLPHENYL)BENZENESULFONAMIDE SHARING AN UNREPORTED BINDING SITE WITH TCF-4 AT THE β-CATENIN ARMADILLO REPEATS DOMAIN AS ANTICANCER AGENT

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BACKGROUND-AIM

The Wnt/β-catenin signaling pathway appears to be dysregulated in tumor initiation, proliferation, and metastasis in several type of cancers as colon, lung, pancreatic and ovarian cancer. In the last decades, intensive efforts have been documented to discover specific Wnt/ β-catenin signaling pathway inhibitors, but no inhibitors have been approved for the clinical treatment of cancer. We synthesized novel N-(heterocyclylphenyl)benzenesulfonamides as β-catenin inhibitors and tested them on Wnt/ β-catenin-dependent cancer cell lines.

METHODS

We identified potential Wnt/β-catenin inhibitors through structure-based virtual screening. Cell viability was evaluated by XTT or MTT colorimetric assays. 30 x 103 cells/well were seeded in 96-well plates and exposed to increasing concentrations of different compounds (range 0-300 μM) for 48 or 72 h. The luciferase report assay of Topflash/Foplash was used to measure the activity of Wnt/β-catenin signaling pathway. The Topflash/Fopflash vector and plasmid pTK-renilla together were co-transfected to the cells. The cells were treated with each β-catenin inhibitor for 12 h and then the luminescence intensity was measured. In co-immunoprecipitation assays 2 × 104/cm2 HCT116 cells were transfected with pcDNA/Myc, TCF4 and cotreated with 50 mM LiCl and 50 μM compound for 24 h. In the in vivo xenograft 1 × 108 HCT116 cells/mL were inoculated subcutaneously into BALB/Cnu/nu mice and treated with intraperitoneal injections of 100 μL compound (25 mg/kg) every 2 days.

RESULTS

We synthesized new N-(heterocyclylphenyl)benzensulfonamides derivatives as inhibitors of the β-catenin signaling pathway with a robust interaction with β-catenin. In crystallographic studies of the β-catenin armadillo repeats domain, compound 9 superimposed to Tcf-4 highlighting a common binding site within the hotspot binding region close to Lys508, thus validating the prediction of the crystallographic studies. In co-immunoprecipitation study compound 9 abrogated the association between β-catenin and TCF-4. Compound 9 strongly inhibited luciferase activity, induced in vitro cell death in SW480 and HCT116 cells and inhibited in vivo tumourigenicity of a human colorectal cancer line HCT116.

CONCLUSIONS

We described the synthesis and antitumor activities of novel N-(heterocyclylphenyl)benzenesulfonamide β-catenin inhibitors. Our data highlight the potential of this novel class of β-catenin inhibitors as anticancer agents and pave the way for further development.

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A MULTIOMICS APPROACH TO DECIPHER THE MOLECULAR LANDSCAPE OF CARDIAC MYXOMA

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BACKGROUND-AIM

Cardiac myxoma (CM) is a common primary neoplasm of the heart, that despite being biologically benign represents a life-threatening condition due to high embolization potential. Protein Kinase cAMP-Dependent Type I Regulatory Subunit Alpha (PRKAR1A) is currently the only known pathogenic gene for CM, whose inactivation underlies familial CM cases. However, only 3-10% of CMs represent familial disease and most cases arise sporadically due to somatic PRKAR1A mutations or other yet unknown molecular aberrations. Our study aimed to determine molecular hallmarks characterizing sporadic CMs by means of next-generation sequencing technologies.

METHODS

Nucleic acids were extracted from 27 FFPE-embedded sporadic CM tumors, adjacent normal or blood samples collected between 2014 and 2022. A multi-omics approach combining whole exome sequencing, CpG DNA methylation arrays, transcriptome, and small non-coding RNA (sncRNA) profiling coupled with integrative data analysis was applied to dissect the molecular mechanisms of CM pathogenesis.

RESULTS

Genomic profiling of tumoral and adjacent normal or peripheric blood samples allowed us to perform genome-wide determination of somatic variants present in neoplastic tissue and detect 7 tumors bearing pathogenic PRKAR1A mutations. Comparison of transcriptional, CpG methylation, and sncRNA profiles of normal and tumoral tissues revealed that global perturbations in gene expression are mediated by aberrations in CpG methylation and deregulation of miRNA expression. Interestingly, we found that inhibition of PRKAR1A-regulated cAMP-dependent signaling occurs independently of the tumor genetic background. Principle component analysis showed that PRKAR1A - mutated tumors cluster separately from non-mutated ones and are characterized by enhanced G protein-coupled signaling. Tumors that do not bear PRKAR1A mutations are characterized by elevated expression of mast cell markers indicating enrichment of this cell population in this tumor group.

CONCLUSIONS

These findings outline a complex molecular and functional landscape of CMs, highlighting novel players and possible disease biomarkers, worth to be exploited in the future.

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ADIPOCYTE-DERIVED SECRETOME INDUCE A CDK4/6 INHIBITOR-RESISTANT PHENOTYPE IN HORMONE RECEPTOR-POSITIVE BREAST CANCER CELLS

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BACKGROUND-AIM

The use of Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i), such as Palbociclib, is currently a standard of care for breast cancer (BC) patients with advanced hormone receptor-positive (HR+)/HER2− disease. However, intrinsic and acquired resistance to CDK4/6i is common, and the underlying mechanisms are not fully understood. Considering the adverse effects of obesity on BC progression, here we explored the impact of adipocyte secretome on sensitivity of BC cells to CDK4/6i treatment.

METHODS

We used fully differentiated 3T3-L1A cells (model of white adipocytes), and human HR+ MCF-7 cells along with Palbociclib-resistant (PalboR) counterpart. MCF-7 cells were incubated with 3T3-L1A-conditioned media (CM) in the presence or not of Palbo and cell viability, cell cycle, colony formation, and motility assays were performed. The half-maximal inhibitory concentration (IC50) values were calculated by GraphPad Prism 7. A comparative bioinformatic analysis was conducted on the transcriptome of BC cells co-cultured with 3T3-L1A-CM and PalboR cells to evaluate putative common mediators of resistance to CDK4/6i.

RESULTS

Palbo reduced cell viability in a dose-dependent manner in MCF-7 cells but in a lesser extent in 3T3-L1A-CM treated cells. IC50 of Palbo was significantly higher in the presence of CM (7 μM vs 0.7 μM in untreated cells). Palbo was unable to affect colony formation and motile phenotype in CM-treated cells. Cell cycle analysis revealed that Palbo was also unable to arrest cells in the G1 phase in the presence of 3T3-L1A-CM. The comparison of the transcriptomes of 3T3-L1A-CM-treated MCF-7 and PalboR cells revealed 195 common upregulated genes. These genes are involved in biological processes related to endoplasmic reticulum stress, cell migration and motility (STRING software). Of note, heat shock protein 70 (HSP70), the product of HSPA5 gene, showed the highest node degree in the protein-protein interaction network, suggesting its potential involvement in 3T3-L1A-induced PalboR phenotype.

CONCLUSIONS

Our data revealed a novel role for adipocyte-derived secretome in inducing a CDK4/6i resistant phenotype in HR+ breast cancer cells. This understanding may allow to identify new potential biomarkers and/or targets to overcome CDK4/6i resistance, especially in obesity setting.

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HEPARAN SULPHATE PROTEOGLYCANS IN OVARIAN CANCER AGGRESSIVENESS AND CHEMORESISTANCE: THE ROLE OF EXT1

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BACKGROUND-AIM

Exostosin glycosyltransferase 1 (EXT1) is an enzyme involved in the elongation of the saccharide chain of heparan sulfate proteoglycans (HSPGs). HSPGs are important components of the extracellular matrix (ECM) and the cell surface. HSPGs consist of repeating disaccharide units with a variable and dynamic degree of sulfation that create binding sites for positively charged amino acids in cytokines, growth factors and ECM components. These factors can trigger the activation of various downstream signaling pathways which promote cancer cell aggressiveness.

METHODS

We analyzed EXT1 protein levels by immunohistochemistry in a tissue microarray of ovarian cancer patients and investigated the correlation with patient outcome using the Kaplan–Meier plotter.
We examined the distribution of HSPG in ovarian cancer cells by cytofluorimetry to select cell lines in which EXT1 could be overexpressed (MDAH-2774) or silenced by shRNAs targeting the Ext1 gene (OVCAR-429). Treatment with the enzyme heparinase was used to cleave the HSPGs on the cell surface. We analyzed the expression profile of these cell lines by transcriptomics and GSEA analysis. The migration ability was analyzed by time-lapse microscopy and RT/PCR immunoblot analysis of EMT markers. Annexin V and MTT assays were used to check cell viability after drug treatment.

RESULTS

We found that EXT1 expression is upregulated in high-grade serous ovarian cancer with platinum resistance and is associated with poor prognosis. We have developed cell line models showing that ectopic expression of EXT1 in epithelial cells is sufficient to increase HPSGs levels, whereas silencing of the Ext1 gene has the opposite effect. We observed that the expression of HSPGs modulates the EMT properties of ovarian cancer cells. Cells with reduced EXT1 expression had low migration and invasion ability, which is consistent with the GSEA analysis showing lower enrichment of the EMT category. In addition, cells with increased EXT1 expression showed lower sensitivity to platinum drugs, which could be restored by heparinase treatment. All these features could be due to the effect of HSPGs on signaling through the Signal Transducer and Activator of Transcription 3 (STAT3) and MAP kinases.

CONCLUSIONS

Overall, our results suggest that EXT1 modulates the EMT program through the biosynthesis of HSPGs. This effect can be attributed to the regulation of STAT3 signaling and may underlie the aggressiveness of ovarian cancer cells.

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INSIGHTS INTO THE ROLE OF KCTD15 IN THYROID CARCINOGENESIS

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BACKGROUND-AIM

KCTD15 belongs to the potassium channel tetramerization-domain containing family involved in various biological processes, including proliferation, apoptosis, differentiation and metabolism, as well as in the pathogenesis of several human diseases including cancers. Thyroid cancer (TC) is the most common endocrine tumor, and its prevalence is constantly rising. Novel biomarkers and therapeutic targets for this disease are needed. In this study, we explored the role of KCTD15 in TC carcinogenesis.

METHODS

The gene expression profile of the KCTD15 in human tissues was investigated in The Cancer Genome Atlas Thyroid Cancer (TCGA-THCA) by using several bioinformatic tools. Western blot and q-RT-PCR were carried out to confirm the altered expression of KCTD15 in a panel of TC cell lines. Functional experiments were conducted to assess the biological role of KCTD15 on the behavior of TC cells.

RESULTS

In silico analysis revealed that KCTD15 was downregulated in papillary thyroid tumor (PTC) samples compared to non-cancerous thyroid tissues. This analysis revealed also that the BRAF-like PTC samples presented significant lower expression of KCTD15 compared to RAS-like PTC samples. Interestingly, KCTD15 downregulation correlated with tumor progression. Restoring KCTD15 expression led to a reduction in the proliferation rate of PTC cell line compared to control transfected cells.

CONCLUSIONS

Our preliminary results suggest that KCTD15 may play a role in thyroid carcinogenesis and could potentially serve as a biomarker for this malignancy.

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INVESTIGATION ON A SOLUBLE FORM OF THE P2X7R (SP2X7R) AS A USEFUL INDEX IN VARIOUS INFLAMMATORY DISEASES

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BACKGROUND-AIM

The P2X7R is a receptor for extracellular ATP expressed on the plasma membrane of various immune cells. The P2X7R is a potent stimulant for NLRP3 inflammasome activation and cytokine release and participates in the pathogenesis of different inflammatory states and diseases, including infective, cardiovascular, metabolic, neurologic, and cancer-associated inflammatory conditions.
Considering that we recently identified a soluble circulating form of P2X7R (sP2X7R) that correlated with inflammatory indexes, such as C reactive protein (CRP), in the present study we extended the investigation to various inflammatory conditions.

METHODS

By ELISA we firstly measured the circulating levels of sP2X7R in healthy subjects of various ages, to better establish the reference values. Subsequently, we quantified sera levels of sP2X7R in pathologic conditions such as COVID-19, in physio pathologic states, such as pregnant women across the three trimesters of pregnancy, and in samples from patients with ocular diseases.

RESULTS

The examination of sera samples from COVID-19 patients with various degrees of disease severity showed that most patients, besides increased inflammatory and coagulative indexes, IL-6 and IL-10 included, presented augmented sP2X7R and sNLRP3. Notably, sP2X7R significantly correlated with some inflammatory markers, disease severity and adverse clinical outcome.
Analysis of maternal sera samples showed that sP2X7R levels were: i) significantly higher in pregnant women at the first trimester of pregnancy than in control age-matched not pregnant women; ii) increasing across the three trimesters of pregnancy; iii) elevated in early pregnancy in overweight and obese women compared with those with normal BMI.
sP2X7R was also detectable in aqueous humour and vitreous humour. The comparison of sP2X7R levels in healthy conditions and in various ocular or systemic pathological conditions with ocular inflammatory involvement, such as: glaucoma, Fuchs endothelial dystrophy, pseudoexfoliatio, AMD, diabetes mellitus and retinal detachment, showed that the sP2X7R might also be a marker of ocular inflammatory status.

CONCLUSIONS

In conclusion, our observations indicate that the sP2X7R is present in human plasma/serum and other body fluids. Its possible role as pro-inflammatory mediator with a function in disease spreading and amplification, needs to be investigated. sP2X7R increased levels in some inflammatory conditions suggest that it could be a useful biomarker in diagnostic medicine.

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INVESTIGATING THE ROLE OF CES1 IN METABOLIC REPROGRAMMING OF ACUTE MYELOID LEUKEMIA

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BACKGROUND-AIM

Metabolic reprogramming is a hallmark of cancer. Cancer cells undergo metabolic adaptation in response to low-nutrient microenvironments. This ability is essential for tumor cell survival and progression. Recently, carboxylesterase 1 (CES1) has been identified in colorectal carcinoma (CRC) as an essential NF-κB-regulated lipase promoting metabolic adaptation and tumor progression. Specifically, the lipase CES1 promotes cancer cell survival under energy stress conditions in CRC, by increasing TAG breakdown to fuel fatty acid oxidation and preventing their toxic build-up. As CRC, other cancers such as Acute Myeloid Leukemia (AML), depend on oxidative metabolism. Therefore, we investigated whether CES1 could play a key role in AML metabolic adaptation.

METHODS

Public datasets of AML patients were analyzed for CES1 expression. A panel of five AML cell lines was tested for CES1 levels both in basal (BC) and energy stress conditions (ES). 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 analysis of patient datasets showed that CES1 expression is higher in M4 and M5 FAB AML subtype and relates to worse prognosis in AML patients. We showed that AML cell lines cultured under ES exhibit increased levels of CES1. In addition, the pharmacological blockade of CES1 by the commercially available inhibitor GR-148672X results in reduced survival and increased cell death in all the analyzed cell lines.

CONCLUSIONS

Metabolic adaptation is a novel hallmark of leukemogenesis required for tumor initiation, progression, and therapeutic responses. Our preliminary data suggest that CES1 could be involved in governing the metabolic requirements of AML cells.

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LACTATE WREAKS HAVOC ON NK CELLS INTENT ON FIGHTING BREAST CANCER

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BACKGROUND-AIM

Once considered a waste product of glycolysis or fermentation, recent findings on the new roles of lactate have changed our knowledge. Lactate is now recognized as a signalling molecule and fuel source for cancer cells in a glucose-restricted environment. It controls multiple functions of immune cells,thus being an indicator of poor prognosis in many cancers.Its presence in the tumor microenvironment (TME),for instance, leads to the polarization of immunosuppressive phenotypes of dendritic cells and impairs the cytotoxic capabilities of T lymphocytes, thus representing a potential obstacle to the efficacy of immunotherapies.Here we sought to analyze the effect of lactate derived from breast carcinoma on the number, phenotype and function of NK cells.

METHODS

To evaluate the effect of lactate on NK cell function, NK cells isolated from peripheral blood of healthy human donors were treated with the supernatant of lactate-producing tumor cells or the lactate recombinant form. Functional assays such as proliferation, apoptosis, cytotoxicity and degranulation were performed by coculturing lactate-treated NK cells with MDA-MB-231 and MCF-7 breast cancer cell lines and analysis by flow cytometry. The ability of NK cells to migrate toward breast cancer spheroids was investigated using a microfluidic device fabricated by standard soft lithography methods and analyzed by fluorescence microscopy.

RESULTS

After sublethal lactate treatment, NK cells slowed their proliferative rate without undergoing massive apoptosis. Flow cytometric analyses showed that lactate-exposed NK cells significantly reduced the expression of activating molecules such as CD69, GZMB and IFNγ, and activating receptors such as NKp46, NKp30 and NKp44. Moreover, lactate-treated NK cells showed a reduced cytotoxic capacity against breast cancer target cells as evaluated by degranulation assay.
Migration experiments on microfluidic devices showed a reduced ability of NK cells to migrate towards lactate-treated tumor spheroids compared to controls.
Further experiments are underway to pharmacologically block the lactate pathway and thereby reverse the immunosuppressive phenotype of NK cells.

CONCLUSIONS

These results may provide a rationale for evaluating molecular strategies aimed at targeting lactate production in breast cancer TME in order to boost NK cell functions, thus positively impacting the NK cell-based immunotherapy.

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BIOINFORMATIC ANALYSIS OF PROTEIN CARGO OF MULTIPLE MYELOMA-DERIVED EXTRACELLULAR VESICLES FOR DISCOVERY OF NEW PROGNOSTIC BIOMARKERS

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BACKGROUND-AIM

Multiple Myeloma (MM) is a hematological malignancy characterized by pathological interaction of plasma cells with bone marrow tumor microenvironment. Extracellular vesicles (EVs) shed by MM have been reported to be involved in disease progression delivering molecular signals. Thereby we hypothesized that their molecular composition may reflect the disease stage and include prognostic biomarkers detected by liquid biopsy to avoid invasive procedures. Here we investigated whether MM-derived EVs could be a potential source of biomarkers.

METHODS

A proteomic profile of OPM2 MM cell line and their relative EVs was analyzed by high-resolution mass spectrometry LC-MS/MS approach. Results underwent an enrichment bioinformatic analysis (EnrichR) and were further validated on a proprietary RNAseq data collection of MM patients (Neri and Gutierrez) and the CoMMpass RNAseq database. Finally, the candidate proteins were validated on the peripheral plasma of MM patients using the ELISA.

RESULTS

We found 2549 proteins in OPM2 cells and 865 in OPM2-EVs, of which 732 were common to both and 133 were exclusively enriched in OPM2-EVs. The selective enrichment of protein in cells or EVs was confirmed by Western blot.
The bioinformatic analysis on the DisGeNET database revealed a wider enrichment in cancer–related pathways for vesicular in comparison to cellular proteins, strengthening the rationale to search for cancer biomarkers in the EV compartment. 21 gene expression profiles of the most EV enriched proteins in the cancer-related datasets were compared through different stages of disease on a proprietary RNAseq dataset. Only MIF, PHB and RPS6 were significantly overexpressed in MM patients compared to healthy donors.
Their clinical relevance evaluated on the CoMMpass RNAseq database of MM patients, using Kaplan-Meier survival curve and COX univariate regression analyses showed that the expression of MIF, PHB and RPS6 might be prognostic factors for OS and PFS. The proteins were further validated by ELISA of MM patients' plasma. The obtained results indicated that MIF levels in the plasma of smoldering MM and MM patients were significantly higher compared to those of healthy donors.

CONCLUSIONS

The data show that MIF a promising EV-related prognostic biomarker.

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ANTIBIOTIC-RESISTANT E. COLI CAN MODULATE RENEWAL/DIFFERENTIATION SIGNALS OF THE COLON MUCOSA: FOCUS ON THE WNT/β CATENIN PATHWAY

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BACKGROUND-AIM

Nowadays, antibiotic resistance is a major public health issue, with an increasing number of pathogens becoming capable of eluding conventional Antibiotic therapies.
This becomes crucially relevant when applied to symbiotic bacteria such E. coli, a typical symbiont of the colonic mucosa.
The relation between E. coli and antibiotic resistant E. coli and its possible modulation on pathways of proliferation and differentiation hasn't been explored widely.
Our study aims at evaluating how E. coli and Carbapenemase E. coli may affect renovation/differentiation signals of the colonic mucosa, with a focus on the WNT/β catenin pathway.

METHODS

Colonic epithelial cells, Caco-2, were treated in the proliferation phase (3 d.o.) and differentiation phase (16 d.o.) with graded dilutions of bacteria in E. coli (ATCC 35218) and E. coli carbapenemase (ATCC 25922) culture broth, selected from current literature. We assessed epithelial cell viability by MTS; WNT-β catenin signaling modulation of gene expression by RT-PCR. Cell cycle and extracellular vesicles (EVs) analysis were also performed.

RESULTS

Exposure of colon epithelial cells to the broth of the two E. coli strains caused a modulation of cell viability and mitochondrial metabolism, as well as the production of EVs. Gene expression of molecules downstream of the WNT signal, such as APC, LEF1, JUN/AP1, CXCL8, CDND1 and cMYC, was significantly different.
Furthermore, in proliferating cells the effects of exposure to E.Coli antibiotic-resistant were significantly different than in differentiated cells.

CONCLUSIONS

In this study, we observed that antibiotic-resistant E. coli products can modulate the WNT/β-Catenin signal of colon epithelium renewal. Further clinical and experimental observations will be necessary to obtain a clearer picture of the interactions between antibiotic-resistant E. coli and the pathophysiology of the colon epithelium.

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FOCUSED ULTRASOUNDS AS A NEW METHOD FOR THE RELEASE AND IDENTIFICATION OF PUTATIVE BIOMARKERS IN PANCREATIC CANCER

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BACKGROUND-AIM

Pancreatic cancer (PC) is one of the most aggressive tumors, typically characterized by late-stage diagnosis and poor outcome. MicroRNAs and proteins released from the tumor microenvironment into body fluids/bloodstream, represent promising non-invasive biomarkers for early cancer detection. Here, we took advantage of an innovative ultrasounds (US)-based instrument (SonoWell, InnoSol) to treat PC cells in order to promote and amplify the release of molecules with the aim to identify novel putative diagnostic PC biomarkers.

METHODS

Three human pancreatic adenocarcinoma cell lines (T3M-4, Panc-2 and Paca44) and a non-cancerous pancreatic epithelial line (HPanEPic), were treated with SonoWell instrument. Control cells (incubated at the same conditions, without sonication) were included in the analysis.
MicroRNAs release in the supernatants were profiled by TaqMan-based qRT-PCR microfluidic cards (Thermo Fisher), while proteins were analyzed by Antibody Arrays (Human Apoptosis Array C1, Human Cancer Discovery Array C3 and NF-kappa B Pathway Screening Array – RayBiotech).
Publicly available datasets of circulating miRNAs in PC patients were also interrogated (dbDEMC).

RESULTS

The expression levels of 22 miRNAs in T3M-4 cells, 11 miRNAs in Panc-2 and 22 miRNAs in Paca44, none of which identified in non-cancerous cell line profiling, were increased in the supernatant of US-treated cells compared to control cells (RQ>2).
Among miRNAs released and common to at least two tumor cell lines, the expression levels of miR-320a, miR-32-5p, miR-339-3p and miR-502-3p, were also significantly up-regulated in serum of PC patients compared to controls.
Preliminary data of proteins released after sonication, identified approximately thirty factors more expressed in the supernatant of treated tumor cells than in non-treated controls (fold increase ≥1,5, signal increase ≥50%).

CONCLUSIONS

We demonstrated that US-mediated sonoporation amplifies the release of several miRNAs and proteins in the analyzed PC cell lines, highlighting the use ultrasound to identify candidate circulating biomarkers.
This study makes novel molecules available to be considered as non-invasive biomarkers in PC, encouraging further studies aimed at deepening their role in PC and to support validation of their expression levels in the sera/plasma of PC patients.

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NOTCH-MEDIATED MODULATION OF MIRNA CARGO IN MULTIPLE MYELOMA-DERIVED EXTRACELLULAR VESICLES VIA NOTCH PATHWAY INHIBITION

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BACKGROUND-AIM

The interaction between multiple myeloma (MM) and the bone marrow microenvironment is pivotal for tumor progression, prominently involving the Notch pathway. Extracellular vesicles released by MM cells (MM-EVs) serve as carriers of essential molecular signals, including proteins and miRNAs, playing a significant role in this pathological communication. This study aims to investigate how inhibiting the Notch pathway influences the miRNA cargo of MM-EVs, shedding light on its implications for MM progression.

METHODS

OPM2 cells were modified to express shRNA targeting NOTCH2 (OPM2N2KD), Jagged1 and Jagged2 (OPM2J1/2KD), or a scrambled sequence and validated by western blot. Nanoparticle tracking analysis (NTA) assessed EV size/concentration. MiRNA profiling of MM-EVs conducted on 754 miRNAs identified modulated miRNA, subsequently validated by qPCR. Pathway enrichment and network analyses identified targets of deregulated miRNAs and their pathways.

RESULTS

NTA showed that NOTCH2 inhibition did not affect MM-EV size/concentration. MiRNA profiling revealed significant deregulation in miRNA expression in EV from OPM2N2KD and OPM2J1/2KD. qPCR validation confirmed that miR-766, miR-216, miR-331, and miR-505 were downregulated in both EVs and producer cells. Pathway and network analyses identified 199 genes targeted by these miRNAs, linked to 20 pathways related to cell cycle and adhesion. KEGG analysis highlighted involvement in various cancer-related pathways. The Molecular Complex Detection (MCODE) algorithm identified significant modules from the PPI network, revealing key genes implicated in DNA repair, consistently with MM genomic instability. Additionally, genes involved in drug resistance and disease progression, such as SOCS1 and AMBRA1, were identified.

CONCLUSIONS

This study provides insights into the molecular mechanisms underlying MM progression and highlights potential avenues for developing novel therapeutic strategies. Inhibition of the Notch pathway in MM cells affects the miRNA cargo of EVs, potentially influencing cellular processes and signaling pathways involved in MM pathogenesis. The identified miRNAs and associated pathways could serve as targets for further investigation and possible therapeutic interventions in MM.

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ANDROGEN RECEPTOR AND TRPM8: DO THEY INTERPLAY TO MODULATE BREAST CANCER CELL BEHAVIOUR?

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BACKGROUND-AIM

Breast cancer (BC) is a heterogeneous disease with different molecular subtypes, each showing different behavior, outcome and response to therapy. The androgen receptor (AR)-signalling influences BC growth/progression but AR-dependent changes differ among BC subtypes: AR expression correlates with increased disease-free survival and is a positive predictive biomarker of response to endocrine therapy in ER+ BCs; AR is mainly considered oncogenic in triple negative BC (TNBC), instead.
Recently, TRPM8, a promising target for several type of cancers, has been reported as an AR-regulated gene in prostate cancer and to be over-expressed in BCs. However, the exact role played by TRPM8 in BC, its possible association with BC subtypes and/or AR expression and its suitability as a target for BC therapy are largely unexplored.
Here we investigated if androgen/AR-signaling might modulate TRPM8 expression and function assessing the consequence of AR/TRPM8 functional interplay in different experimental models of BC.

METHODS

ER+ luminal A and B (MCF-7; ZR75) cells and their tamoxifen-resistant counterparts; triple-negative MDA-MB-231 BC cells. UALCAN data analysis Portal, qRealTime PCR; WB; colony/spheroid formation assay; wound-healing/ transmigration/ assays.

RESULTS

AR and TRPM8 expression was queried using the breast invasive carcinoma TCGA data set. AR and TRPM8 show an opposite expression patterns in the different breast cancer subtypes, which appears to be particularly evident in both ER+ luminal and Triple negative breast tumors. Similarly, an inverse trend in the level of AR and TRPM8 can be observed when analyzing the different subset of TNBC, e.g. TNBC basal-like and TNBC-LAR (expressing AR similarly to the ER+ luminal subtype).
Accordingly, TRPM8 mRNA levels are very low in MCF7 and increase in the more invasive ZR-75 and in the more aggressive MDA-MB-231. Androgen administration reduces TRPM8 mRNA content in both MCF7 or ZR-75 cells. It also decreases cell ability to form either colonies or spheroids as well as cell motility. All these effects were more evident in presence of TRPM8 antagonists.

CONCLUSIONS

Our preliminary results suggest the existence of an AR/TRPM8 functional interplay that may be regarded as a potential new therapeutic target for BCs.

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ANALYSIS OF GLP1-R FUNCTION AND TRAFFICKING IN THE EARLY STAGE OF PDAC TUMORIGENESIS UPON DYSMETABOLIC CONDITIONS

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BACKGROUND-AIM

The Glucagon-like peptide receptor (GLP1-R) is a G protein-coupled receptor recognizing GLP-1 ligand, an incretin hormone secreted by the intestine, which regulates insulin secretion, satiety induction, glucagon inhibition, and appetite suppression. GLP1-R agonists are well-established anti-diabetic drugs, mimicking incretin effects in treating type 2 diabetes (T2D) and metabolic disorders, major risk factors for various cancers, including pancreatic ductal adenocarcinoma (PDAC). PDAC is a highly aggressive cancer with a low survival rate, poor clinical outcomes, and resistance to chemotherapy. Despite the clinical significance of GLP1-R, its role in metabolic syndrome and PDAC development is poorly understood. The present study investigates GLP1-R mechanisms and trafficking in the early stages of PDAC development under dysmetabolic conditions.

METHODS

PDAC development upon dysmetabolism was investigated in: 1) MITO-Luc-LSL-Kras (G12D/+); Pdx-1-Cre (MKC) mice model fed with high-fat diet (HFD) to mimic dysmetabolism-induced alterations; 2) HPDE-K-RasG12V (Human Pancreatic Ductal Epithelial Cells) cultured with high glucose and free fatty acid levels to mimic metabolic alterations.

RESULTS

We demonstrated that GLP1-R protein levels are significantly down-modulated in both in vivo and in vitro models upon dysmetabolic conditions. Since metabolomics analysis detected low levels of succinyl-CoA in HFD mice, we evaluated global succinylation level in our experimental models. Of note, western blotting analysis and immunoprecipitation revealed a global decrease in succinylated protein levels upon metabolic alteration, including GLP1-R protein. In this light, we rescued GLP1-R levels boosting succinylation, by desuccinylase SIRT5 inhibitors, and blocking the autophagic flux, by the lysosomal inhibitor Bafilomycin A1. Interestingly, the rescue of GLP1-R levels or activity affects HPDE-K-RasG12V cell proliferation and migration, preventing their aggressiveness upon dysmetabolic conditions.

CONCLUSIONS

Impaired GLP1-R signaling plays a pivotal role in the early stages of PDAC progression. Increasing GLP1-R succinylation might stabilize its function, counteracting tumorigenesis. These results highlight novel therapeutic targets for PDAC patients affected by metabolic syndrome.

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EVALUATION OF PLANT RIBOSOME-INACTIVATING PROTEIN TOXICITY ON INTESTINAL CELLS

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BACKGROUND-AIM

Ribosome-inactivating proteins (RIPs) are well-known plant toxic enzymes, which are able to irreversibly block cell protein synthesis, causing the consequent cell death. Recently, several new RIPs have been purified from edible plants. Despite the large amount of literature on RIPs, little information is available on the impact of these enzymes on the intestinal epithelium. In order to set up the experimental conditions to be used with toxic proteins from edible plants, saporin was chosen as preliminary model because it is one of the best known and most studied RIPs.

METHODS

Saporin cytotoxicity was evaluated in dose- and time-response experiments on Caco-2 and HT-29 intestinal epithelial cell lines (derived from colon adenocarcinoma). Cell viability was assayed by evaluation of metabolic activity and by vital dye exclusion. The involvement of apoptosis or necrosis was determined by flow cytometric analysis through double staining with annexin V/propidium iodide. The apoptotic cell death was confirmed by the estimation of caspase 3/7 activation through luminometric assays. The involvement of necroptosis and oxidative stress was also evaluated in RIP-treated cells.

RESULTS

Cytotoxicity experiments demonstrated that, in both cell lines, saporin strongly reduced cell viability after 72 h (EC_{50}s in 10-100 nM range), with about 90% of dead cells after treatment with 1 μM saporin. Apoptosis resulted the main cell death pathway triggered by RIPs. Indeed, 1 μM saporin was able to induce apoptosis in about 70% of treated cells after 24 h. No necrosis involvement was observed. The discrimination between apoptosis and necrosis is important as apoptosis does not cause inflammation. A low, but significant protective effect of ROS scavengers and apoptosis/necroptosis inhibitors was reported in saporin-treated cells, demonstrating also the involvement of oxidative stress and necroptosis.

CONCLUSIONS

Our preliminary results give us useful information to analyse the impact of RIPs from edible sources on intestinal diseases.

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TARGETING CANCER METABOLISM: HOW TO TURN OFF OXIDATIVE METABOLISM OF OVARIAN CANCER (OC) CELLS THROUGH CES1 INHIBITION

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BACKGROUND-AIM

Cancer cells can metabolically adapt to facilitate their growth, invasiveness, and metastatic potential. Thus, metabolic mediators are potential druggable targets, especially when cells develop resistance to standard treatment that is one of the crucial reasons that makes OC the deadliest gynaecological cancer worldwide. Several evidence sustain that fat availability plays a crucial role in promoting aggressiveness and chemoresistance in OC. In this context, CES1 is a NF-kB-regulated lipase able to fuel FAO by enhancing TAG breakdown and its metabolic relevance in cancer progression was depicted for the first time in the colorectal carcinoma (CRC).
Alike CRC, OC shows addiction to both lipid metabolism and NF-kB and the tendency to preferentially metastasize toward the peritoneal cavity and the omentum, a fat-rich organ. These features make OC attractive to investigate whether CES1 could represent a promising target to counteract OC metabolic adaptation and overcome therapy-resistance.

METHODS

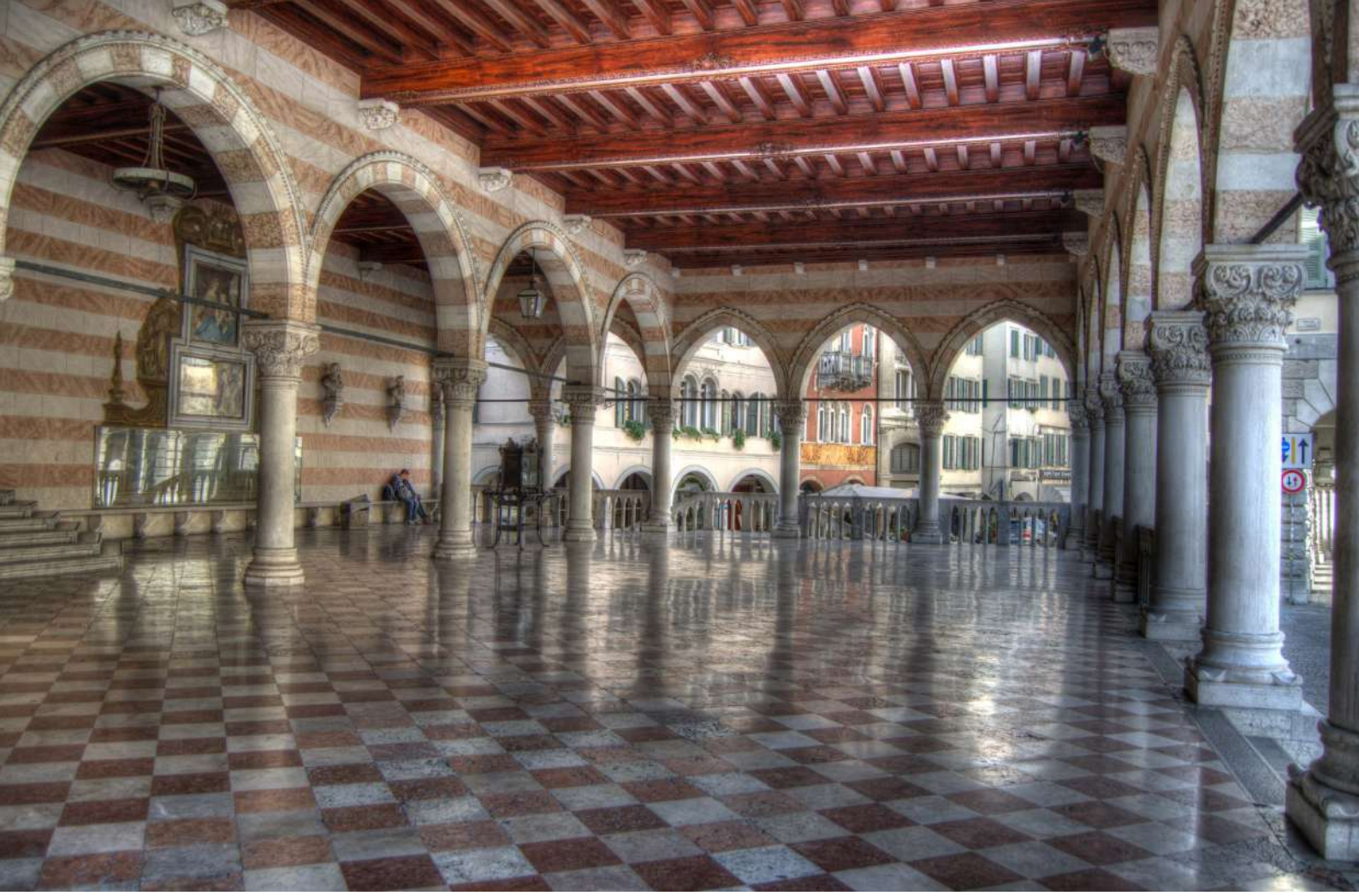
We selected a panel of 10 OC cell lines established from different histological subtypes (mucinous, low-grade serous, clear cell, and high-grade serous OC) and primary cells to culture in energy stress (ES) conditions, to reproduce the metabolic reprogramming to OXPHOS occurring in the nutrient depleted TME.

RESULTS

We demonstrated that OC cells become resistant to platinum when cultured under ES, increasing the reliance on OXPHOS and FAO, and upregulating CES1. This suggests that lipid metabolism and CES1 could be relevant during nutrient fluctuations and chemoresistance. Indeed, unlike carboplatin, CES1 blockade by GR-148672X inhibitor impairs the switch to OXPHOS, blocks autophagy flux and is effective in killing OC cells. Interestingly, CES1 inhibition is contextual specific for the oxidative switch of cancer cells in ES, which occurs in TME but not often in physiological contexts. Moreover, we found that the co-expression of CES1 and CPT1A, the rate limiting enzyme of FAO, is associated with worse disease specific survival in refractory/resistant OC patients.

CONCLUSIONS

These data underscore that CES1-driven metabolic reprogramming is mostly relevant during the switch towards OXPHOS and sustain the actionability of CES1 inhibition in platinum-resistant OC cells in the era of precision medicine.



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