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ABSTRACT BOOK

P001

METAINFLAMMATION MODIFIES PHOSPHATIDYLCOLINE (PC) AND PHOSPHATIDYLETHANOLAMINE (PE) RATIO IN ADIPOSE TISSUE AND PLASMA OF CARDIOVASCULAR DISEASES PATIENTS (CVDS) PROMOTING CARDIAC REMODELLING TROUGH NLRP3 INFLAMMASOME

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BACKGROUND-AIM

NLRP3 inflammasome is the result of obesity-induced metainflammation but the mechanisms by which obesity can metabolically activates NLRP3 is not fully understood. Recently, it is demonstrated that perturbation in cardiac lipidome promotes left ventricle (LV) remodelling though the release of bioactive lipids that play a key role in cell stability and inflammation. In this regard, we focused our attention on phosphatidylcoline (PC) and phosphatidytlethanolamine (PE) species as lipid trigger of both NLRP3 inflammasome activation and maladaptive LV remodelling.

METHODS

40 patients from IRCCS Policlinico San Donato were unrolled and their epicardial adipose tissue (EAT)and plasma were drawn during surgery. LV geometry was evaluated by echocardiography and the clinical data were collected. PC and PE species were quantified by ESI-MS/MS using Quattro Ultima triple-quadrupole mass spectrometer methods. NLRP3 plasma level were quantified using ELISA assay. All the data were analysed using R version 3.3.1.

RESULTS

Top 10 differential expressions of PC and PE species in EAT and plasma is observed subdividing CVDs patient according to HOMA and ADIPO Q indexes ($p<0.05$). PC/PE ratio in plasma correlates with the main cardiometabolic parameters associated to LV remodelling including fasting glucose, HOMA, ADIPO Q indexes and with the circulating levels of NLRP3. NLRP3 in plasma correlates with parameters of metainflammation including total fatty acid ($p<0.02$), fasting glucose ($p<0.0001$), triglycerides ($p<0.01$), acid uric ($p<0.0061$). Negative correlations were found between NLRP3 circulating level and PC/PE ratio, a newer parameter of LV decline ($p<0.0003$) and between PC/PE and relative wall thickness, an echocardiographic index of LV remodelling ($p<0.05$). PC/PE ratio decreases in patient with worst LV remodelling contrarily NLRP3 circulating level increases ($p<0.05$ in concentric remodelling).

CONCLUSIONS

Our data suggest that metainflammation promotes perturbation in local and circulating level of bioactive PC and PE species accompanied to the increase of NLRP3 inflammasome. Moreover, LV decline is characterized by with a reduction of circulating PC/PE ratio contrarily to NLRP3 increase in plasma of CVDs patients with concentric remodelling.

P002

A NOVEL SEVERE FORM OF PEDIATRIC MALIGNANT VENTRICULAR ARRHYTHMIA: THE TRIADIN KNOCK-OUT SYNDROME

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BACKGROUND-AIM

Triadin is a protein expressed in cardiac and skeletal muscle that has an essential role in the structure and functional regulation of calcium release units and excitation–contraction coupling. Mutations in the triadin gene (TRDN) have been described in different forms of human arrhythmia syndromes characterized by malignant arrhythmogenic phenotype, including "Triadin KnockOut Syndrome" (TKOS). TKOS mainly affects young patients in the childhood, that are often refractory to conventional treatment, substantiating the need to identify new therapeutic strategies to prevent or treat cardiac events. So far, pathogenic mechanism underlying the malignant phenotype has yet to be completely defined and the purpose of our study was to characterize it, generating the first TKOS animal model in Zebrafish (Danio rerio), since its heart is highly comparable to the human heart in terms of functions, signal pathways and ion channels, representing a good model to study cardiac disorders.

METHODS

Exploiting trio whole-exome sequencing approach, we performed the genetic analysis of a severe TKOS form of a 2-year-old boy, who was resuscitated from sudden cardiac arrest with a positive family history.
Then, we generated the first zebrafish model for trdn loss-of-function, by means of trdn morpholino injections, and characterized its phenotype.

RESULTS

After the exome analysis, our group identified the causative variant of the index case: a novel homozygous missense variant (p.L56P) in the TRDN gene.
Then, we assessed the spatio-temporal expression of trdn during zebrafish embryo development and we generated a zebrafish model of trdn loss-of-function by means of morpholino (MO) injection. We characterized skeletal muscle and heart phenotype in trdn-MO-injected embryos, and we observed a dysregulation of cardiac function in terms of a reduction of heart beats. Such a defect in the heart rate was partially recovered by the injection of the wild-type form of the human TRDN and treatment with conventional arrhythmic drugs.

CONCLUSIONS

Based on our results, we propose Zebrafish model as a platform to characterize specific TRDN mutations and their pathogenic mechanisms and to perform high-throughput drug screening in zebrafish to identify candidate drugs for improving treatment for TKOS patients.

P003

IDENTIFICATION OF NEW CANDIDATE GENES ASSOCIATED WITH BRUGADA SYNDROME BY WHOLE EXOME SEQUENCING

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BACKGROUND-AIM

Brugada Syndrome (BS) is an inherited arrhythmogenic disease with risk of sudden cardiac death in young asymptomatic adults. SCN5A is the only known causative gene, though 22 genes have been associated with BS susceptibility; nevertheless, 70% patients still remain genetically undiagnosed. To identify new candidate genes, we performed Whole Exome Sequencing (WES) of 96 patients, both sporadic and familiar cases.

METHODS

WES was performed on NovaSeq Illumina Platform (mean coverage: 180X, 97% target region >20X). Reads were analyzed exploiting Dragen BioIT Platform, coding and splice regions variants (MAF≤0.01%) prioritized and classified according to ACMG guidelines with the support of eVai-EnGenome software. In addition, burden test with Fisher exact test allowed us to extrapolate genes with higher mutation burden, suggesting a possible pathogenic role.

RESULTS

WES analysis showed rare prioritized variants located in about 400 genes. Subsequent burden test allowed to focus on a smaller number of genes, confirming the role of SCN5A and highlighting possible association of genes encoding proteins involved in muscle contraction, development and differentiation, as also suggested by Enrichment analysis with Panther tool.

CONCLUSIONS

Preliminary data identified new candidate genes suggesting a role for structural proteins in disease pathogenesis. These results should be further investigated and confirmed in a larger cohort, also evaluating possible copy number variations. Genotype-phenotype correlations will be performed to better stratify patients, also taking into account also the putative oligogenic inheritance of the disease and evaluating the possible role of multiple variants in the clinical phenotype.

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P004

HIF-1A STABILIZATION REGULATES INFLAMMATION AND PROMOTES REPAIR IN CHRONIC GRANULOMATOUS DISEASE

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BACKGROUND-AIM

Chronic granulomatous disease (CGD) is a genetic disorder caused by mutations in the proteins forming the NADPH complex, which results in defective production of reactive oxygen species (ROS), increased susceptibility to infections and a hyperinflammatory response. Besides participating in direct microbial killing, the generation of ROS during infection is accompanied by local oxygen consumption that results in a condition of inflammatory hypoxia, with stabilization of the hypoxia inducible factor-1 (HIF-1)α and resolution of inflammation. Since the discovery of HIF-1α, several studies on the hypoxia signaling pathway have demonstrated that HIF-1α stabilization displays a protective effect during acute conditions. Based on these premises, we asked whether pharmacological stabilization of HIF-1α could ameliorate inflammation in CGD. To this purpose, we resorted to Thymosin β4 (Tβ4), an active peptide with 43 amino acids known to regulate wound healing, inflammation and tissue regeneration, that has been shown to stabilize HIF-1α in tumor microenvironment.

METHODS

To evaluate whether HIF-1α stabilization by Tβ4 could be therapeutically exploited in CGD, we resorted to monocytes from CGD patients and $p47^{phox-/-}$ mice that have been infected with Aspergillus fumigatus or subjected to a murine model of colitis.

RESULTS

We found a lower expression of HIF-1α in monocytes from CGD patients challenged with Aspergillus conidia and in $p47^{phox-/-}$ mice infected or subjected to murine colitis. Interestingly, Tβ4 administration restored HIF-1α levels in CGD and induced the up-regulation of hypoxia-responsive genes involved in the angiogenesis and repair, thus contributing to reduce inflammation and impair granuloma formation both in pulmonary aspergillosis and colitis.

CONCLUSIONS

The protective effect of HIF-1α stabilization by pharmacologic intervention has been supported by numerous in vivo studies and recent human clinical trials. However, modulating the HIF pathway, particularly in chronic disease conditions, is often considered complex. In CGD, the ability of Tβ4 to activate physiologic HIF-1α suggests that targeting the hypoxia signaling pathway could be therapeutically exploited to regulate inflammation and promote repair in CGD.

P005

USE OF APE1 PROTEIN REDOX INHIBITOR APX2009 IN INFLAMMATORY-BASED INTESTINAL DISEASES

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BACKGROUND-AIM

Inflammatory Bowel Disease (IBD) is an immune-mediated relapsing-remitting chronic disorder characterized by alterations in the intestinal microbiome, defects in mucosal barrier defense, and aberrant innate and adaptive responses. The mechanisms which contribute to the etiology of IBD are complex and not yet fully elucidated. Therapeutic strategies in IBD primarily function by modification of the immune response using anti-TNFα, anti-IL-12, anti-IL-23, and integrin blockers which limit the migration of leukocytes to the gastrointestinal (GI) tract. Recently, it has been demonstrated the involvement of apurinic/apyrimidinic endoribonuclease 1 (APE1) in the IBD. APE1 is a multi-functional protein that acts as a DNA repair protein and as a transcriptional coactivator coordinating, through a redox-based mechanisms, the cellular response to oxidative stress, regulating angiogenesis, inflammation, and proliferation. Previous studies have demonstrated increased APE1 redox activity in tissues removed from IBD patients, indicating underlying oxidative stress within the GI tract. APX3330 is a selective APE1 redox inhibitor leading to a decrease in the activity of pro-angiogenic and pro-inflammatory transcription factors such as HIF-1α and NF-kB to modulate VEGF, TNFα, and other inflammatory cytokines. Interestingly, it has been demonstrated that APX3330 alleviates intestinal dysfunction in a mouse model of spontaneous chronic colitis. Here, we characterized a human IBD colon cell model treated with anti-inflammatory agents, focusing on the effect of second-generation molecules of the drug APX3330, named APX2009.

METHODS

HCT116 cell line was stimulated with TNFα in the presence or absence of the APE1 redox inhibitor APX2009 and the drug's efficacy was evaluated focusing on the inflammatory phenotype and on the underlining molecular mechanisms.

RESULTS

APX2009 blocks TNFα-induced activation of IL-8 production in a dose-dependent manner without affecting cell viability. Interestingly, APX2009 and Infliximab, a chimeric monoclonal antibody neutralizing TNFα through direct binding, have a synergic effect on TNFα-induced IL-8 gene expression inhibition.

CONCLUSIONS

This work is suggestive of the use of APE1 redox inhibitor as a novel strategy for the treatment of inflammatory-based intestinal diseases.

P006

PPAR-ALPHA AND PPAR-GAMMA INVOLVEMENT IN GRADE-DEPENDENT LIPID STORAGE OF CLEAR CELL RENAL CELL CARCINOMA CELLS

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BACKGROUND-AIM

The cells of clear cell Renal Cell Carcinoma (ccRCC), the most common and lethal subtype of renal cancer, are rich in lipid droplets reflecting a lipid metabolism reprogramming with an increment of fatty acid synthesis and decrement of their oxidation. Grade-dependent alterations in fatty acid metabolism were described in ccRCC and we showed grade-dependent lipid storage in ccRCC tissues and primary cell cultures (PCC). Here we investigated the involvement of PPARα and γ and their transcriptional targets CPT1A and ACLY in the molecular mechanisms driving the grade-dependent lipid storage in ccRCC PCC. The effect of specific PPARα and γ agonists and antagonists on viability/proliferation of low- and high-grade ccRCC PCC has been also evaluated.

METHODS

PCC was established from Fuhrman low- and high-grade ccRCC and normal cortex tissue samples. Gene Ontology (GO) enrichment analysis performed on published ccRCC PCC transcriptome. Neutral lipid storage in PCC evaluated by western blot analysis of lipid droplet marker PLIN2. PPARα and γ expression were evaluated by western blot, CPT1 and ACLY transcript expression by Real-Time PCR. The effect of PPARα and γ agonists and antagonists on ccRCC PCC viability was assessed by MTT assay.

RESULTS

PPAR signaling pathways were lipid metabolism GO terms significantly enriched in ccRCC PCC. The expression of PPARα and CPT1A was significantly downregulated in ccRCC versus normal cortex, and upregulated in high-grade PCC showing low lipid storage. Conversely, the expression of PPARγ was significantly upregulated in ccRCC and particularly in low-grade PCC showing high lipid storage. ACLY expression analysis is in progress. PPARα and CPT1A expression are inversely correlated whereas PPARγ is directly correlated with lipid storage in ccRCC PCC. Preliminary data showed that treatment of low-grade ccRCC PCC with a PPARα antagonist or PPARγ agonist reduced cell viability.

CONCLUSIONS

Our data show a grade-dependent modulation of PPARα and γ expression in ccRCC PCC that correlates with intracellular lipids, suggesting PPARs functional involvement in lipid storage. PPARα inhibition and PPARγ activation with specific drugs affect the viability of low-grade ccRCC cells suggesting a putative grade-specific metabolic targeted therapy in ccRCC.

P007

FGFR2C/PKCε AXIS AS A PROMISING MOLECULAR TARGET TO COUNTERACT THE MALIGNANT FEATURES IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS

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BACKGROUND-AIM

The pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy whose main characterizations are KRAS activating mutations and high aggressive phenotype. Based on our recent findings demonstrating that the high aberrant expression of the mesenchymal isoform of FGFR2 (FGFR2c) in PDAC cells activates PKCε, which in turn controls receptor-mediated epithelial to mesenchymal transition (EMT), we investigated the involvement of these signaling events in the establishment of additional tumorigenic features.

METHODS

Stable transfection with specific shRNA has been performed in PANC-1 and Mia PaCa-2 PDAC cell lines, expressing divergent levels of the FGFR2c, to establish the impact of FGFR2c or PKCε depletion on different tumorigenic features. Scratch assay, Matrigel pre-coated in vitro Boyden chamber and Soft agar tests were applied to assess the effects on cell migration, invasion and anchorage-independent growth, respectively. The possible impact of FGFR2c/PKCε signaling shut-off on the cross talk between cell survival and invasion was assessed checking cell viability by clonogenic assay and monitoring the amounts of cleaved PARP1 and MCL-1, as well as SRC phosphorylation by biochemical approaches.

RESULTS

Our results showed that only when highly expressed, FGFR2c triggers PKCε downstream signaling, responsible for the invasive response of PDAC cells to FGF2 and for anchorage-independent growth. FGFR2c/PKCε axis is also involved in cell viability, as well as in the control of the interplay between cell survival and invasion, possibly via MCL-1/SRC-mediated reorganization of actin cytoskeleton.

CONCLUSIONS

Overall, our results strongly suggest that the FGFR expression profile significantly contribute to PDAC tumor cell plasticity, determining the responsiveness to paracrine FGFs and consequently the possible efficacy of FGF/FGFR/PKCε axis-based target therapies. In addition, being PKCs RAS-independent substrates, the identification of PKCε as hub molecule downstream FGFR2c at the crossroad of signaling networks governing the main malignant tumor hallmarks could represent an important advance towards innovative target therapies overcoming RAS.

P008

HIGH-THROUGHPUT SERUM PROTEOMICS AND CLINICAL DATA ANALYSIS VIA SUPERVISED LEARNING CORRELATION DISCOVERY APPROACHES IDENTIFIES PROGNOSTIC BIOMARKERS IN SARS-COV-2 INFECTION

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BACKGROUND-AIM

The global pandemic disease COVID-19 is challenging due to the heterogeneity of both individual susceptibility to infection and disease severity. Furthermore, the persistence of long-term sequelae demands for better characterization of COVID-19 pathophysiology and for the identification of potential biomarkers of disease outcome. Proximity extension assay (PEA) allows the simultaneous detection and quantification of hundreds of proteins using low amount of serum. To date, only few comprehensive studies identifying sera biomarkers correlating with COVID-19 disease severity have been published but are limited to very small patient cohorts.

METHODS

160 COVID-19 patients were enrolled, of whom 80 had a “non-severe” form of the disease and 80 had a “severe” outcome. Sera were collected at hospital admission and analyzed by PEA technology to assess 276 protein biomarkers associated with inflammation, cardiometabolic, and neurologic diseases. The main clinical and hematochemical data associated with disease outcome were collected together with serological data to form a dataset for the supervised machine learning techniques which acted as a reference point for a set of correlation discovery approaches which have been employed in order to identify potential correlations with the target variable representing the outcome of the patients.

RESULTS

we identified 9 proteins (i.e. CD200R1, MCP1, MCP3, IL6, LTBP2, MATN3, TRANCE, α2-MRAP, and KIT) that contributed to the correct classification of COVID-19 disease severity, when combined with relative neutrophil and lymphocyte counts. Of these, 3 are associated with pathologies of the central nervous system. Importantly, we also found protective factors, that are downregulated in the most severe cases.

CONCLUSIONS

By combining PEA with statistical methods that can handle many variables, even in the presence of a relatively small sample size, we identified 9 potential serum biomarkers of a “severe” outcome. The role played by most of the biomarkers, emerging from our analysis, was confirmed by available literature data, further supporting the solidity of the overall approach. Notably, we demonstrated for the first time that markers of neurological diseases are strong predictors of COVID-19 severity.

P009

EXTRACELLULAR VESICLES AS PROGNOSTIC BIOMARKERS IN SARS-COV-2 INFECTION

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BACKGROUND-AIM

Few studies have investigated the possible role of extracellular vesicles (EV) in the host response to SARS-CoV2 infection. For this purpose, we assessed whether cytofluorimetric assays that both enumerate their presence in plasma and evaluate their phenotype could have a diagnostic role in COVID-19 patients.

METHODS

101 COVID-19 patients were enrolled, of whom 51 had a "non-severe" form of the disease and 50 had a "severe" outcome. Platelet poor plasma was collected at hospital admission and analyzed by flow cytometry to assess and quantify the presence of small (≈200nm in size), intermediate (200-500nm) and large EVs (≈1µm). Vesicles were analyzed for the expression of markers of endothelial, platelet, leukocyte, NK-cells, mural cell, and neural cell origin (i.e. CD31, CD34, CD42b, CD45, CD140b, CD56, and N-Cadherin). The main clinical data associated with disease outcome were collected as well.

RESULTS

Patients with adverse outcome were older, obese and more frequently had atrial fibrillation. The total number of EVs, especially the smaller, intermediate and larger ones was lower in patients with severe prognosis. Conversely, the fraction of EV expressing CD140b and CD56 as well as the quantity of CD31+ and CD56+ EV per µL of plasma was higher in severe patients. N-Cadherin had an opposite behavior. Univariate logistic regression confirmed that age, obesity, atrial fibrillation, and the frequency of N-Cadherin+ EV conferred an increased risk for severe outcome. Conversely, small EVs were associated with a non-severe outcome. Multivariate logistic regression showed that age and small EV enumeration were the only independent predictors of disease severity (O.R., 95% CI: 1.07128, 1.023358-1.121447 and 0.9967324, 0.9939587-0.9995139 respectively).

CONCLUSIONS

EV are independent predictors of COVID-19 severity. However, the fact that they could be associated with both positive and negative outcomes suggests a complex, cell specific, biological relevance for this way of intercellular communication.

P010

INCREASE OF DONOR-DERIVED CELL-FREE DNA (DD-CFDNA) IN CORONARY ALLOGRAFT VASCULOPATHY IN HEART TRANSPLANTED PATIENTS

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BACKGROUND-AIM

Cardiac Allograft Vasculopathy (CAV) is the leading cause of death of patients that survived the first year after Heart Transplant (HTx) and increases as a function of time after transplant. Cell-free DNA is released by dying cells, so that donor-derived cell free DNA (dd-cfDNA) could be employed as a marker of graft injury in solid organ transplants. Therefore, we reasoned that dd-cfDNA could reflect ongoing subclinical graft rejection, and may be useful to monitor graft status in stable HTx patients.

METHODS

99 HTx patients in the last 10 years were enrolled. In 46 of these, blood samples were analyzed by NGS to identify dd-cfDNA. Massive miRNA analysis was conducted in a subset of 11 subjects (4 CAV; 4 no-CAV and 3 healthy controls) to characterize transcriptional signatures. Troponin T (hs-TnT) and NT-proBNP were measured by automated immunoassays. Echocardiographic and coronary angiogram data were collected.

RESULTS

HTx recipients were divided into 2 groups: half of the patients had CAV, while the other half not (no-CAV). No significant differences between the two populations were observed, aside from time from HTx and prevalence of chronic kidney disease. A significant difference in dd-cfDNA fraction was found between CAV and no-CAV (p=0.001) with mean values of 0,44% and 0,14%, respectively. Furthermore, while NT-proBNP levels were higher in CAV patients (p=0.034), hs-TnT ones were not (p=0.076). Markers of hypertrophy and diastolic dysfunction were also significantly different (p=0.021 and p=0.034, in CAV e no-CAV). Last, we observed by univariate logistic regression that time from transplant, hs-TnT, and dd-cfDNA were significant predictors of CAV occurrence with an OR, 95% CI of 1.009691, p=0.001; 1.021162, p=0.027 and 1.182722, p=0.035. Circulating miRNA profile was characterized by a signature that clustered CAV from non-CAV patients.

CONCLUSIONS

dd-cfDNA is a strong predictor of CAV development. The latter, that, as expected, increases with time from transplant, is also associated with diastolic dysfunction and chronic kidney disease. Moreover, the molecular profiling of circulating miRNA may provide additional information mostly for the identification of new potential markers to predict disease evolution.

P011

PDL-1 MODULATION BY UPA/UPAR SYSTEM: IMPLICATIONS FOR IMMUNE CHECKPOINT INHIBITOR THERAPY

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BACKGROUND-AIM

Over the last years, immunotherapy has revolutionized cancer therapy. Immune checkpoint inhibitors (ICI), such antibodies against PD-L1 and PD-1, have shown effectiveness against a large number of cancer types, including melanoma and non-small-cell lung cancer. This response includes durable remissions in many patients who had previously failed multiple other therapeutic strategies. However, even in these cancers, only 10%–30% patients respond to anti-PD-L1/PD-1 therapy.
Hence, development of a new strategy is required for improvement of therapeutic efficacy in melanoma and other malignancies. Emerging evidences suggest that improved prognosis and clinical outcomes in melanoma patients subjected to anti-PL-1 therapy is associated with high PDL-1 positivity. Recently, Tseng et al. showed that targeting Plasminogen Activator Inhibitor (PAI-1) by its inhibitor tiplaxtinin synergizes with anti-PDL-1 checkpoint blockade in a model of murine melanoma, thus paving the way for a more effective melanoma treatment. The membrane-associated plasminogen activation system (urokinase-type plasminogen activator, uPA; uPA receptor, uPAR; uPA inhibitor type-1, PAI-1) is actually considered one of the main systems involved in tumor invasion and metastasis. Here, we propose to inhibit PDL-1 endocytosis targeting LRP-1/uPAR and or /PAI-1 complex.

METHODS

PAI-1 and uPAR silencing by siRNA and uPAR/LRP-1 complex inhibition by specific antibodies were performed to block PD-L1 endocytosis and increase PD-L1 membrane levels. Surface PD-L1 expression was analyzed by flow cytometry and confocal immunofluorescence.

RESULTS

We demonstrated that in A549 (non-small cell lung cancer cells) and in A375M6 (metastatic melanoma cells), PAI-1 and uPAR silencing by siRNA, or uPAR/ LRP-1 complex inhibition by specific antibodies, are able to block the PD-L1 internalization and, consequently, to increase PD-L1 membrane levels on cancer cells resulting then more sensitive to anti PD-1/PD-L1.

CONCLUSIONS

Our data demonstrated that uPAR silencing or uPAR/LRP-1 inhibition result in a significant increase in surface PD-L1 levels opening the way for new combined therapeutic strategies with anti-PD-1/PD-L1. These findings have significant implications for immunotherapeutic approaches to cancer therapy.

P012

CD32 DRIVES AUTOANTIBODY-ENHANCED TROGOCYTOSIS TO FACILITATE HIV-1 INFECTION OF RESTING CD4 T CELLS

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BACKGROUND-AIM

The identification of biomarkers expressed selectively on the surface of resting CD4 T cell subsets harboring replication-competent HIV-1 would facilitate the targeting and elimination of the latent viral reservoir in infected individuals. The Fcγ receptor CD32 has been reported to serve as such a biomarker for resting CD4 T cells, but its role has remained controversial.

METHODS

To study this process, we established a CRISPR/CAS9 co-culture model using primary macrophages and CD4 T cells as well as a cell line-based donor-target model system. Transient expression of CD32 isoforms, chimeras and mutants, other surface receptors as well as the addition of modulating antibodies provided unprecedented mechanistic insight into trogocytosis.

RESULTS

Thanks to this system we could show that CD32 is not expressed de novo by HIV-1-infected CD4 T cells, but is acquired from CD32-positive macrophages by trogocytosis in an infection-independent process. CD32 drives the transient, cell contact-dependent transfer of distinct plasma membrane patches and associated receptors, including CD32 itself, but also HLA-DR, CD209 and chemokine receptors. Trogocytosis is enhanced by antibodies bridging CD32-positive donor cells and CD4 target T cells and, intriguingly, a subset of patients with chronic HIV-1 infection harbor T cell-reactive IgG autoantibodies with this capacity. Importantly, HIV-1 particles preferentially bind to distinct macrophage-derived, CD32-positive plasma membrane patches on resting CD4 T cells, resulting in increased virion fusion to and infection of these viral reservoir cells. Trogocytosed surface receptors also confer additional migration and adhesion properties to CD4 T cells that may shape an infection-prone environment.

CONCLUSIONS

Thus, trogocytosis can result in a transient plasticity of the surface proteome of immune cells with CD32 as a regulator and marker. In HIV patients, this process can enhance HIV-1 susceptibility of resting CD4 T cells and may thus contribute to expanding the latent HIV-1 reservoir in vivo.

P013

CHOLESTEROL-FREE KETOGENIC DIET FEEDING IMPROVES EXPERIMENTAL NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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BACKGROUND-AIM

Non-alcoholic fatty liver disease (NAFLD) is recognized as the most common liver disease worldwide. It encompasses a spectrum of pathological conditions from simple steatosis to non-alcoholic steatohepatitis (NASH) which can further progress to cirrhosis and lead to hepatocellular carcinoma (HCC). Despite great advances in understanding the pathophysiology and identifying therapeutic targets, nevertheless, there is not an approved therapy for NAFLD. Lifestyle changes, including diet, are, so far, the most effective interventions in NAFLD. Recently, low carbohydrates ketogenic diets (KDs) have been increasingly used for weight loss. However, the efficacy of KDs in improving NAFLD is controversial due to contradictory data obtained in animal experiments. In this study, we investigated the capacity of a cholesterol-free KD to improve NAFLD in mice.

METHODS

NAFLD was induced in C57BL/6 mice by feeding with a cholesterol-enriched Western Diet (WD) for 16 weeks, followed by switching animals to KD for up to eight weeks.

RESULTS

KD administration increased ketone body production while reduced body weight and liver weight by upregulating the gene expression of PPARGC1 and decreasing the hepatic content of triglycerides. Moreover, KD restored the physiological capacity to metabolize glucose, as testified by the glucose tolerance test (GTT). In addition, the liver proteomic analysis further confirmed an improvement in glucose metabolism and insulin resistance in KD-fed mice. KD administration ameliorated transaminase release together with the histological severity of steatosis and necro-inflammation. Mice receiving KD showed a lowering in the hepatic expression of pro-inflammatory/-fibrogenic markers such as CCl2, IL-12, CD11b, α1-procollagen, TGF-β, OPN and Gal-3 which were accompanied by a significant reduction in hepatic monocyte-derived macrophage (MoMFs) infiltration and fibrosis. Moreover, intestinal microbiota analysis showed that NASH improvement was associated with a recovery of bacterial composition to conditions similar to those of healthy mice.

CONCLUSIONS

Altogether these results indicate that a cholesterol-free ketogenic diet is effective in improving metabolic derangements and steatohepatitis and might represent a potential therapeutic strategy for NAFLD.

P014

WOUND HEALING OF CYSTIC FIBROSIS EPITHELIUM IS ACCELERATED BY HUMAN MENSENCHYMAL STEM CELLS AND TAILORED CFTR MODULATORS

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BACKGROUND-AIM

Cystic fibrosis (CF) airways are affected by a deranged repair of the damaged epithelium resulting in altered regeneration and differentiation. Previously, we showed that human amniotic mesenchymal stem cells (hAMSCs) corrected base defects of CF airway epithelial cells via connexin (CX)43-intercellular gap junction formation. In this scenario, it is unknown whether hAMSCs can operate a faster repair of a damaged CF airway epithelium through a CX43-mediated mechanism and if the triple combination of CF Transmembrane Conductance Regulator (CFTR) modulators (Trikafta™) can accelerate wound repair.

METHODS

A tip-based scratch assay was employed to study wound repair in monolayers of CFBE14o- cells (CFBE, homozygous for the F508del mutation). hAMSCs were either co-cultured with CFBE cells before the wound or added to the wounded monolayers at 6, 24, and 48 hours. As a positive control, NIH-3T3 fibroblasts (CX43+) were added to wounded cells. HeLa cells (CX43-) were used as negative controls. γ-irradiation was optimized to block CFBE cell proliferation. A specific siRNA was employed to downregulate CX43 expression in CFBE cells. Trikafta™ was added during wound repair.

RESULTS

CFBE cells showed a delayed repair as compared with wt-CFTR cells (16HBE41o-). hAMSCs enhanced the wound repair rate of wounded CFBE cell monolayers, especially when added post wounding. γ-irradiation of CFBE cells slowed wound repair, indicating that their proliferation contributes to wound closure. hAMSCs and NIH-3T3 fibroblasts, but not HeLa cells, increased wound closure of irradiated CFBE monolayers. CX43 downregulation accelerated CFBE wound repair rate without affecting cell proliferation. Trikafta™ accelerated wound closure rate, in particular at earlier times (6 h).

CONCLUSIONS

We conclude that hAMSCs and fibroblasts enhance the repair of a wounded CF airway epithelium, likely through a CX43-mediated mechanism mainly involving cell migration. Tailored CFTR modulators show promise for the acceleration of wound repair in the context of a CF airway epithelium.

P015

CCRL2 EXPRESSION BY ALVEOLAR CAPILLARY CELLS REGULATES NK CELL LUNG IMMUNE SURVEILLANCE

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BACKGROUND-AIM

Cancer related inflammation is sustained by inflammatory mediators and leukocyte infiltration. Leukocyte recruitment is mediated by chemokines and chemokine receptors (CKR). C-C Chemokine Receptor-Like 2 (CCRL2) is a CKR-like receptor devoid of the ability to activate signaling and cell migration. When expressed on the surface of endothelial cells (EC), CCRL2 binds chemerin, a non-chemokine chemotactic protein, and promotes leukocyte recruitment, as observed for NK cells in lung tumors.

METHODS

To characterize the expression of CCRL2 by lung ECs, mouse and human scRNA datasets from healthy and lung tumor specimens were quired (E-MTAB-7458, GSE160876, GSE165063). ChIP-seq (SRA030934.1), LUAD transcriptomic and epigenetic datasets were retrieved from GDC.

RESULTS

This analysis identified the selective expression of CCRL2 by an EC subset named “generally capillary cells” (gCap), both in mouse and human cells. The number of gCAPs was heavily reduced in lung tumor ECs with the few gCaps populating lung tumors expressing the oncogenic transcriptional factors (TF). Conversely, healthy gCaps were enriched in homeostatic TFs like GATA2. ChIP-seqs analysis identified GATA2-binding sites in proximity of CCRL2 open reading frame. Notably, in vivo treatment with the VEGFR inhibitor (PTK787) restored gCap CCRL2 expression. CCRL2 downregulation was supported by hypermethylation of the gene, compared to normal tissue. In agreement with this observation, the treatment of the lung cell line 1G11 with Decitabine (AZA) increased CCRL2 expression.

CONCLUSIONS

We have identified gCaps as a lung EC subset that selectively expresses CCRL2. These cells are located at the alveoli-air interface, where they exert a pathogen sensing role. In lung tumors this population is strongly reduced in parallel with CCRL2 downregulation. Normal gCap phenotype was partially restored by VEGFR inhibitors. These results identify CCRL2 as a NK cell lung homing molecule that might be exploited to promote NK cell-mediated lung immune response.

P016

CHARACTERIZATION OF INFLAMMATORY PARAMETERS OF SICILIAN SEMI AND SUPERCENTENARIANS

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BACKGROUND-AIM

Super-centenarians are people who have reached 110 years. This age is achieved by about one out 1,000 centenarians, whereas semi-supercentenarians are persons aged 105-109 years. Research on their morbidity found that they remain free of major age-related diseases until the end of life when they die of exhaustion of organ reserve. Thus, these ultracentenarians are a great model of healthy ageing. Their characteristics of delayed onset of age-related inflammatory diseases and compression of morbidity suggest an optimal control of immune-inflammatory responses. We extend previous studies by carrying out further analyses of the inflammatory parameters in a Sicilian cohort.

METHODS

Fifty-four Sicilians, aged 19 and 111 years, were investigated. Participants were divided into four groups: Adult, age range 19-64; Older, age range 65-89; Long-lived individual (LLI), age range 90-104; Ultracentenarians ≥105 years old. The serum levels of C-reactive protein (CRP), interferon-gamma (IFN-γ) and interleukin-6 (IL-6) were measured as well as blood neutrophil lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR).

RESULTS

IL-6 and CRP serum levels significantly increase with age (IL-6: Adult vs. LLI, p <0.0001; Older vs. LLI, p <0.0001; Adult vs. Ultracentenarians, p=0.0015; Older vs. Ultracentenarians, p=0.0162. CRP: Adult vs. LLI, p=0.0269), then decrease in ultracentenarians, although not significantly. We also observed a trend for IFN-γ levels to increase up to 89 years, and then gradually decrease from ≥90. The NLR increases with age, with the maximum value recorded in Ultra group (2.59 mean). Finally, no significant differences with age were found for the PLR, although Ultracentenarians showed the lowest mean value (102.88), more similar to the Older (103.5) than to the LLI (121.6) group.

CONCLUSIONS

Our results indicate that semi and supercentenarians show a lower value of key inflammatory markers than LLI, particularly for IL-6. These data suggest that ultracentenarians have better inflammation control than LLIs, likely responsible for their lower incidence of age-related diseases. Analysis of the immunophenotype (in progress) of these subjects may give important information on their immune status, completing the overview.

P017

EXPLOITING THE POTENTIAL OF SALIVARY LIQUID BIOPSY FOR THE DETECTION OF HPV IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

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BACKGROUND-AIM

Human papilloma virus (HPV) is a DNA virus capable of infecting mucous. In most of cases, the infection is cleared by the immune system, but a prolonged exposure to HPV can progress to cancer. 40–60% of head and neck squamous cell carcinomas (HNSCC) are linked to HPV, a risk factor especially among young people in industrialized countries. HNSCC are not identified early due to their slowly growth and their locations not easy to see. Despite the prognostic value of HPV, the use of HPV-DNA as a diagnostic marker is not fully developed. HPV-DNA can be detected in saliva specimens of patients with HPV-driven cancers. Considering this, we employed saliva samples to optimize a non-invasive RT-PCR assay for HPV infection and prove the hypothesis that the presence of HPV DNA represents a risk factor for HNSCC. The potential of this work is to highlight how an HPV screening program based on salivary testing can be useful for early cancer detection and patient monitoring.

METHODS

In this study all participants were tested for the presence of HPV in self-collected saliva specimens. Viral DNA was extracted from saliva using an automated instrument. A multiplex RT-PCR was employed for the detection of the 28 most frequent HPV Types (SeeGene). Patients' demographics were collected in a clinical database. Statistics was performed by STATA16, and significance was set at $p < 0.05$.

RESULTS

127 patients and 30 controls were enrolled. Most of patients had a diagnosis of HNSCC (85%), 39% of which involving the tongue. 11,8% resulted positive for HPV DNA in saliva, specifically for high-risk subtypes (50% type 58; 43% types 45,59,39 and 7% type 18). HPV+ patients were compared to those were found negative. HPV infection was related to the TNM stage, especially with pT1N0 ($p \leq 0.001$) and with primary vs relapsed tumors ($p=0.04$). Independent of site, the assay reached a sensitivity and a specificity of 100% and an agreement of 100%, compared to the oropharyngeal swab (Cohen's Kappa=1; n=65).

CONCLUSIONS

Our findings indicate that salivary HPV testing is a non-invasive and convenience test that could become part of routine clinical management for HPV infection of the oral cavity. This test represents an ideal mode of screening of asymptomatic individuals and a long-term monitoring tool for HPV-driven cancer patients.

P018

REDUCING COLLATERAL TOXICITY OF STANDARD THERAPY IN ACUTE MYELOID LEUKEMIAS BY PRESERVING EPITHELIAL BARRIER FUNCTION

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BACKGROUND-AIM

Acute myeloid leukemias (AML) are among the most frequent malignancies in older people. The "golden standard" therapy for AML, i.e. the combination 7+3 (cytarabine + daunorubicin), is accompanied by significant damage to the mucous membranes, especially in the intestine, and a high percentage of infections. In 2017, a liposomal combination of cytarabine + daunorubicin in a synergistic 5:1 molar ratio (CPX-351, Vyxeos®) was approved by the FDA as first-line treatment of therapy-related AML and MRC-AML, after having demonstrated a better overall survival and percentage of remissions in leukemic patients compared to the "7 + 3" arm. The rationale of this study is to elucidate mechanisms underlying the reduced toxicity and lower mortality of CPX-351 compared to the "7 + 3" combination. Our working hypothesis is that the intestinal mucosal barrier, made up by the concerted action of the epithelial lining, the associated immune system and the communities of colonizing microorganisms, could be a major site for the collateral activity of the chemotherapeutic regimens.

METHODS

We first assessed whether the mucosal barrier function could be differently affected by the CPX-351 versus “7+3” regimens, using in vitro cultures of intestinal Caco-2 cells and in vivo murine models. Intestinal barrier integrity and function were evaluated by measuring the permeability to FITC-Dextran assay, the expression of tight junctions by immunofluorescence and RT-PCR, and the production of inflammatory cytokines by ELISA. In the in vivo model, two different routes of administration (intraperitoneal and intravenous) were tested.

RESULTS

We found that, in contrast to the “7+3” regimen, CPX-351 did not affect viability and barrier function of Caco-2 cells at doses ranging from 1 to 100 μM. In agreement with the in vitro findings, CPX-351 did not cause mortality, body weight loss and did not impair intestinal and liver barrier function as compared to “7+3” treatment in epithelial damage in vivo, an activity independent from the route of administration.

CONCLUSIONS

These results suggest that the liposomal formulation may prevent intestinal epithelial barrier damage thus improving the safety profile of CPX-351 in AML. Studies are ongoing to define the contribution of the microbiota to the CPX-351’s beneficial activity.

P019

αVβ6-INTEGRIN LIGANDS CONJUGATED WITH NINTEDANIB REDUCE TGFβ-INDUCED EMT OF HUMAN NON-SMALL CELL LUNG CANCER

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BACKGROUND-AIM

One of the main advantages of precision medicine is the development of agents capable of supporting effective targeted molecular therapy. Indeed, in recent years, the use of targeted molecular therapy has led to the improvement of therapeutic strategies in different types of tumors.
In particular, TGFß, secreted in tumor microenvironment, induces the overexpression of fibronectin receptor αvß6 integrin in lung cancer cells. We introduce three novel synthetized selective αvß6 receptor ligands which have been conjugated with nintedanib, a potent kinase inhibitor used in advanced adenocarcinoma lung cancer treatments. The conjugation of nintedanib with selective αvß6 receptor ligands allows addressing drug activity directly toward cancer cells, avoiding the onset of negative side effects in normal cells.

METHODS

Human lung adenocarcinoma cells (A549 and H1975 cells) were used to evaluate the biological activity of αvß6-nintedanib conjugates. In addition, human melanoma cells (WM266.4) and murine fibroblasts (L929) were used. Chronic myelocytic leukemia cell line K562 was used as negative control.

RESULTS

The three conjugates were capable to inhibit cancer cell adhesion to fibronectin in a dose-dependent manner. The conjugates were internalized by cancer cells mostly through integrin receptor, favoring longer retention of the drug inside the cell. In addition, the conjugates showed significant inhibition of cell growth, reduction of drug resistance, and inhibition of epithelial mesenchymal transition (EMT).

CONCLUSIONS

These results suggest that the αvb6 integrin receptors could represent key preferential access for an efficient targeted molecular strategy in non-small cell lung cancer.

P020

HOST AND MICROBIAL TRYPTOPHAN METABOLIC PATHWAYS IN CYSTIC FIBROSIS

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BACKGROUND-AIM

Cystic fibrosis (CF) is a genetic, autosomal recessive disorder caused by the mutation of a gene that codes for the Cystic Fibrosis Transmembrane conductance Regulator (CFTR), Mutations in CFTR affect the innate epithelial immune function of multiple organs such as respiratory system. In the lung impaired CFTR activity results in exaggerated and ineffective airway inflammation that fails to control bacterial and fungal infections. In this context, the different Tryptophan (Trp) metabolic pathways, and the balance between them, may represent critical factors for microbial growth and for successful host-microbe interactions. Trp is metabolized via three main pathways: the indole pathway occurring in bacteria, the kynurenine (Kyn) and serotonin (5-HT) pathways both occurring in mammalian cells. The indole pathway, via the aryl hydrocarbon receptor (AhR), barrier physiology. The Kyn pathway, downstream the indoleamine 2,3-dioxygenase 1 (IDO1) enzyme, plays a critical role in regulation of immunity and tolerance, whereas the 5-HT pathway can be involved in the control of infection and inflammation.

METHODS

We resorted to in vivo and in vitro models to study the impact of the different tryptophan metabolic pathways in lung infection and inflammation in CF.

RESULTS

We found a low IDO1 protein expression and Kyn/Trp ratio in $Cftr^{-/-}$ mice as compared to wild-type mice infected with Aspergillus fumigatus. Lower production of kynurenines was also observed in HBE cells from patients with CF compared to non-CF control subject on exposure to A. fumigatus conidia, suggesting that a defective IDO1-dependent tolerance may occur in human CF. $Cftr^{F508del}$ mice also had a defective expression of AhR that correlated with defective functional activity, as revealed by the reduced expression of the downstream Cyp1a1 and Cyp1b1 genes and Cyp1b1 protein. Interestingly, we have seen a dysregulated expression of genes along the serotonin pathway in these mice associated with defective microbial handling and cell recruitment.

CONCLUSIONS

Preliminary as they are, our data suggest a distinct, yet complementary, role of the different Trp metabolic pathways in tissue homeostasis and antimicrobial resistance in the CF lung.

P021

EPIGENETIC REGULATION OF BCLAF1 SPLICING MECHANISM IN AML

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BACKGROUND-AIM

Alternative splicing is a fundamental physiological process for the correct regulation of cellular functioning. Dis-regulation of alternative splicing is increasingly associated with cancer development and tumor progression. BCL2-associated transcription factor 1 (BCLAF1) is implicated in a wide range of biological processes and is continuously being investigated due to its convoluted, emerging function in tumorigenesis and drug resistance. Our previous studies shown that BCLAF1 aberrant expression is involved in differentiation commitment and in pharmaco-resistance especially considering acute myeloid leukemia (AML). Since in CRC have been validated two isoforms of BCLAF1, FL and SL, having opposite roles, relatively oncogenic and tumor suppressor we extended the research on the role of these two isoforms in AML and their regulation with epigenetic drugs.

METHODS

PCR, RT-PCR, Western Blot

RESULTS

Our new results indicated that in AML there is an unbalanced expression of two specific BCLAF1 isoforms, found for the first time in CRC. Also in AML our data shown that the FL isoform has an oncogenic role while the SL isoform has a tumor-suppressor function. Treatment of AML cell lines with epigenetic drugs (e.g. Vorinostat) or with DNA methylation inhibitor (Azacitidine) led to a restoration of the balance of these two isoforms, with decrease of FL and increase of SL isoform both at the transcriptional and at the translational level. Moreover, interestingly, after Vorinostat treatment, we also found a transcriptional and translational regulation of SRSF10 (Serine and Arginine Rich Splicing Factor), factor known to be involved in the alternative splicing of BCLAF1. Our brand-new results suggest that BCLAF1 splicing seems to be modulated by both HDAC and DNMT enzyme and therefore by regulation of epigenetic changes in the chromatin status of this gene.

CONCLUSIONS

We speculate about the in-depth understanding of epigenetic regulation on BCLAF1 splicing process could lead to an innovative and targeted treatment and the introduction of more effective drugs for the treatment of AML and other cancer correlated with BCLAF1 aberrant isoforms expression.

P022

PROBIOTIC LACTOBACILLUS STRAINS ATTENUATE OXYSTEROLS-INDUCED ALTERATION OF HUMAN INTESTINAL EPITHELIUM PERMEABILITY IN VITRO

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BACKGROUND-AIM

The intestinal mucosa acts as a barrier to protect the human body from potentially harmful dietary noxious stimulus that can alter gut epithelium functionality mostly by increasing its permeability or causing a shift on microbiota composition, all factors that lead to inflammation. Oxidative stress and inflammation have been linked to the loss of intestinal integrity, a crucial event in the initiation and progression of intestinal disorders such as inflammatory bowel diseases (IBD) and cancer. In this context, the aim of this study was to evaluate the capacity of two extracts from probiotic strains of Lactobacillus (Lactobacillus plantarum 299v® DMS 9843 and DG® CNCMI1572), to protect intestinal epithelium against permeability alterations induced by oxysterols, cholesterol oxidative products which have been reported to act negatively on microbiota composition and to induce local inflammation, and to investigate the mechanism of action in relation to tight junctions (TJ) modulation and intracellular signaling.

METHODS

Monolayers of differentiated Caco-2 cells have been used as in vitro model of intestinal barrier. The alteration of cell monolayers permeability, treated with oxysterols alone or together with the bacterial extract, was evaluated at different incubation times by assessing transepithelial electrical resistance (TEER) and the modulation of TJ proteins occludin, zonulin and JAM-A related to redox-sensitive MAPKs p38 and ERK1/2 activation measured by Western blot.

RESULTS

The two extracts from Lactobacillus spp. probiotics exerted a protective effect on the monolayer permeability shown as restoration of TEER values ($p<0.001$) and TJ expression ($p<0.05$) which were affected by oxysterols and related, at least in part, to the modulation of p38 ($p<0.05$) pathway rather than of ERK1/2.

CONCLUSIONS

Taken together, our results provide evidence on the ability of two probiotics strains of Lactobacillus spp. to protect intestinal cells against the pro-inflammatory effect of oxysterols in vitro. Our data strengthen the link between harmful oxysterols and inflammation and suggest to keep studying probiotics as a useful tool in the prevention and management of intestinal inflammatory diseases.

P023

T-CELL-BASED THERAPIES FOR THE TREATMENT OF INVASIVE PULMONARY ASPERGILLOSIS

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BACKGROUND-AIM

Invasive fungal infections are a major cause of disease and death in immunocompromised patients, including patients undergoing allogeneic hematopoietic stem cell transplant. Despite progress in the development of effective and safe antifungal drugs, in recent years, the increase in fungal drug resistance has raised the need of adjunctive novel approaches to improve patient outcomes. Over the past decade, there have been pivotal developments in adoptive T cell therapy to produce and expand clinically relevant antigen-specific T cell products. Their use in clinical conditions, especially in treating cancer as well as for prevention of life-threatening viral infections in allogeneic transplant recipients, demonstrated safety and clinical efficacy, providing a potential therapeutic option for patients who are unresponsive to standard treatments.

METHODS

Here, in an experimental mouse model of Aspergillus fumigatus infection, we have tested two different T-cell therapies using rapidly expanded fungus-specific T cells or following gene-engineered of A. fumigatus-specific chimeric antigen receptor (Af-CAR) T-cells. Mice were evaluated for fungal burden, cell recruitment in the bronchoalveolar lavage fluid, lung histology and FACS analysis.

RESULTS

Both strategies lead to reduction in the severity of pulmonary infection, by conferring a therapeutic immune response that reduces fungal burden, level of inflammation and survival in neutropenic mice.

CONCLUSIONS

Collectively, our studies illustrates the potential of T-cells therapies to tackle aggressive infectious diseases that are difficult to control with conventional antimicrobial therapy and supports their clinical development to specifically treat lung fungal infections.

P024

A NEW EMERGING ROLE OF NLRP4 INFLAMMASOME, AS A NEGATIVE REGULATOR OF TBK1, DURING TUMOR PROGRESSION: HIGHLIGHTS ON BREAST CANCER CELL LINES

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BACKGROUND-AIM

NLRP4 is a member of the NLR family of cytosolic receptor, which is strongly expressed in several tissues, including placenta, testis, oocytes, spleen, pancreas, bladder, lung, liver, kidney and thymus. This protein acts as a negative regulator by targeting TBK1 for proteasomal degradation, where a specific NLRP4-DTX4 axis is recruited as a key suppressor of TBK1 during the innate antiviral signaling. TBK1 has been shown to have a critical role in tumor development mediated by the oncoprotein KRAS and activating the kinase AKT signaling pathway. It has been speculated that negative regulation of TBK1 by NLRP4-DTX4 axis, may have an important antitumoral role in tissues with NLRP4 expression. This information prompted us to understand the link between inflammation and tumorigenesis, through the investigation of two proteins: NLRP4 and TBK1. Protein level of NLRP4 and TBK1 in breast cancer cells with a particular attention to T-47D cell line, was investigated.

METHODS

In the present study, a control cell line (MCF-10A), and 3 breast cancer cell lines (T-47D, MCF-7 and MDA-MB-231), were cultured. T-47D were transfected with a plasmid to produce an NLRP4 overexpression. NLRP4 before and after transfection was evaluated and compared to TBK1, both at protein level, by Western Blot (WB)

RESULTS

Our results showed that protein level of NLRP4 was higher in control cell line compared to breast cell lines, in particular in T-47D; instead, TBK1 protein presented higher level especially in T-47D. As we expected, T-47D, after increased NLRP4 expression by transfection, revealed lower TBK1 amount.

CONCLUSIONS

This study discloses a new protective role of NLRP4 Inflammasome during tumorigenesis. Supported by this finding, new strategies for the treatment of breast cancer will be developed.

P025

MODULATION OF NLRP4 AND NAIP INFLAMMASOMES ON T24 AND 5637 CULTURE CELLS, TREATED WITH RESVERATROL, AFTER NITROSATIVE STRESS INDUCTION.

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BACKGROUND-AIM

Resveratrol (3,5,4'-trihydroxystilbene, RSV), a non-flavonoid polyphenol, exerts an anti-oxidative, anti-tumor progression and anti-inflammatory function, acting as a scavenger of reactive oxygen (ROS) and nitrogen (RNS) species. The concept of inflammasome was introduced in 2002 when a multimeric protein complexes related to immune response and cancer progression, such as NLRP4 and NAIP, were discovered. The aim of the present work is to understand the effects of RSV on Urinary Bladder cancer (BCa) Transitional Cell Carcinoma (T24) and Grade II Carcinoma (5637) cell line stressed with Sodium Nitroprusside (SNP), a donor of nitric oxide (NO). We analyze the modulation of NLRP4 and NAIP, two inflammasome platforms, known to be altered in urine sediment of patients with BCa.

METHODS

A concentration kinetic study on T24 and 5637 cells was set up to exclude cytotoxic concentrations of SNP and RSV and culture cells were treated with different concentrations and different exposition times, by Flow Cytometer assay. To evaluate the effect of both substances on the expression of NLRP4 and NAIP, cells were treated with SNP or RSV singularly. While, to evaluate RSV potential beneficial outcome, cells were previously treated with SNP and subsequently administered with RSV. Post-transcriptional and post-translational analysis of inflammasomes were conducted by RT-PCR and Western Blotting.

RESULTS

Flow Cytometer assay revealed that SNP did not exert a cytotoxic effect, whereas RSV showed a dose-time dependent cytotoxic effect on BCa culture cells. Modulation of NLRP4 and NAIP mRNA was observed in T24 and 5637 in all treatments performed, with particular emphasis in cells treated with combined substances respect to control cells. Protein analysis revealed that NLRP4 inflammasome maintained high levels of expression, whereas NAIP showed an opposite trend with reduced expression in combined treatments with SNP and RSV.

CONCLUSIONS

The peculiar expression of NLRP4 and NAIP inflammasomes, at post-transcriptional and post-translational levels, suggests that RSV could act as immunomodulatory factor with possible beneficial effects on inflammatory state of BCa cells.

P026

EVALUATION OF THE ALBUMIN QUOTIENT IN PATIENTS WITH SUSPECTED MULTIPLE SCLEROSIS.

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BACKGROUND-AIM

Until today, the only laboratory test used in the evaluation of multiple sclerosis (MS) has been the detection of oligoclonal bands (OCBs).
To discriminate if immunoglobulins detected in the cerebrospinal fluid (CSF) derive from intrathecal synthesis or by simple diffusion from blood, it is important to evaluate the degree of blood-CSF barrier permeability. For this purpose, we evaluated the performance of albumin quotient (QALB) in patients with suspected MS.

METHODS

The Optilite turbidimetric analyser was used to assess CSF and serum albumin (Low Level Albumin assay, The Binding Site Group, Birmingham, UK) in 44 patients with suspected MS referred to the University Hospital of Udine between January and May 2022. The CSF and serum samples were also evaluated for OCBs. QALB was then calculated as follows: (CSF albumin/serum albumin)x100.

RESULTS

To evaluate the degree of blood-CSF barrier damage, we used the following cut-offs: normal up to 0.7%, mild from 0.8 to 2%, moderate from 2.1 to 5%, severe above 5%. MS diagnosis was confirmed in 13/44 patients; all of them tested positive for OCBs and the permeability of the blood-CSF barrier was either normal (10/13) or with a mild degree of damage (3/13). 31/44 patients were diagnosed with other neurological conditions; in 20/31 the blood-CSF barrier permeability was normal, 8/31 had a mild degree of damage and 3/31 had a moderate degree of damage. Among non-MS patients, only one tested positive for OCBs and showed a moderate degree of blood-CSF barrier damage; this patient later received a diagnosis of meningoencephalomyelitis.

CONCLUSIONS

Our data demonstrated that in patients with MS, detection of immunoglobulins in the CSF indicate an intrathecal synthesis with a normal or mildly damaged blood-CSF barrier. In the patient diagnosed with meningoencephalomyelitis, the evaluation of QALB allowed to ascribe the presence of immunoglobulins in CSF to diffusion from blood circulation rather than from intrathecal synthesis. In conclusion, calculation of QALB allows the most accurate measurement of the degree of blood-CSF barrier permeability in disorders characterized by intrathecal immunoglobulin production.

P027

RADIATION THERAPY-DEPENDENT ORAL MUCOSITIS: HOW THE ORAL DYSBIOSIS MAY PREDISPOSE TO OPPORTUNISTIC FUNGAL INFECTIONS

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BACKGROUND-AIM

Oral mucositis (OM) is a common acute side effect of radiation therapy (RT) in head-neck cancer patients (HNCPs). Although clinical protocols advise the use of probiotics to counteract oral dysbiosis in OM, preclinical studies are needed to delineate the mechanisms by which the oral dysbiosis may predispose to OM. We performed an observational prospective study evaluating oral microbiota milieu during head and neck radiotherapy.

METHODS

We recruited HNCPs > 18 years old, undergoing RT alone or concomitant radio-chemotherapy (RT-CHT), +/- surgery. Non-naïve CHT patients who received antibiotic therapy in the two weeks prior to baseline were excluded. Acute toxicity was assessed using the CTCAE 5.0 grading. We monitored oral microbiome and lipidomics in correlation with RT-dependent OM at 0, 7, 15 days from the beginning of the RT-treatment and 1 month, 2 months post-RT treatment, using oral swab for metagenomics and lipidomics (OMNIgene ORAL kit). In addition, to understand the possible therapeutic effects of specific oral commensals we established an in vitro 3D tongue organoid model.

RESULTS

Currently, 23 patients recruited have an average age of 70 years. We performed a preliminary analysis on 17 patients. Median total RT dose to the primary tumor was 66 Gy (interquartile 62-66 Gy). Only 5 patients received cisplatin and 1 patient cetuximab. Metagenomics for 16S and 18S was performed for the first 4 patients (16S Bacteria and 18S Eukaryotes) and community diversity analysis was performed for the prokaryotic and eukaryotic population. Although preliminary, RT-related oral fungal infection was evident during therapy. The oral microbiome show for each patient a specific signature, although profound changes occur during RT. Regarding lipidomics, preliminary results show that the main lipid changes occur in the glicerophospholipid compartment. Organoid exposure to specific oral commensals restores the oral eubiome.

CONCLUSIONS

The present study represents an important tool to better delineate the oral myco/microbiome affected by RT±CHT, eventually leading to new mechanistic insights in advanced targeted therapy or OM prophylaxis protocols in HNCPs.

P028

GENE BINDING BY MIR-223 INDUCES FLOTILLIN-1 EXPRESSION AND FUNCTION TO INCREASE RECEPTOR ACTIVITY AND REGULATE MYELOID DIFFERENTIATION.

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BACKGROUND-AIM

Retinoic Acid (RA) induces myeloid differentiation upregulating miR-223 expression. To investigate novel functions of miRNAs and their role in differentiation regulation, we searched 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. Confocal microscopy, biochemical assays and cell phenotype studies were used to investigate cell biology.

RESULTS

We determined genome-widely in myeloid cells undergoing RA-induced differentiation, gene regulatory sequences bound by nuclear miR-223, characterized by activating (H3K4me3) and/or repressing (H3K27me3) histone marks. Among the complementary sequences bound by miR-223, we selected the flotillin-1 (FLOT1) gene, which displayed the H3K4me3 mark. Flotillin-1 is an essential component of lipid rafts. In a panel of hematopoietic cell lines including HL60 and NB4 and their RA-resistant mutants, we found that flotillin-1 expression is dependent on active RA transduction pathway. In CD34+ hematopoietic progenitors FLOT1 is strongly induced upon stimulation with growth factors inducing proliferation or myeloid differentiation. FLOT1 overexpression in HL60, U937 and THP1 cells increases vitamin D and RA-induced differentiation, whereas its stable knockdown produces the opposite effects. The tyrosine kinase receptor CSF1R interacts with flotillin-1 and is recruited into lipid rafts upon stimulation with CSF1, increasing its signalling activation kinetics. FLOT1 overexpression drives CSF1R to Rab4 endocytic vesicles, increasing receptor recycling to cell membrane after activation.

CONCLUSIONS

miR-223 transcriptionally induces the expression of biologically significant genes. Among them, FLOT1 enhances myeloid differentiation. It potentiates the activity of CSF1R by recruiting it to signalling active lipid rafts and increasing Rab-4 mediated membrane recycling of the receptor.

P029

DOXORUBICIN-INDUCED SENESCENT FIBROBLASTS PROMOTE IN VITRO TUMOUR CELL GROWTH AND INVASIVENESS. QUERCETIN WORKING AS A SENOLYTIC AGENT REDUCES THESE PRO-TUMORAL EFFECTS.

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BACKGROUND-AIM

Cellular senescence (CS) is one of the processes involved in aging and age-related diseases. Many strategies have been employed to reduce the harmful effect of CS, and one of the most promising was the research on senolytic drugs that selectively kill senescent cells. In this study, we have analysed the potential senolytic activity of Quercetin on WI-38 senescent fibroblasts induced by Doxorubicin, a chemotherapy drug used to treat several types of cancer, including osteosarcoma a malignant bone tumour with a high mortality rate due to metastatic spread and therapy toxicity. Moreover, we have also studied the effects of conditioned media from Doxo-induced senescent fibroblasts treated (SQM) and not-treated with Quercetin (SM) on U2OS, an osteosarcoma cell line.

METHODS

WI-38 fibroblasts were induced to senescence with Doxo and then treated with Quercetin 40 μM for 3 days to evaluate its senolytic activity. Cell proliferation, cell cycle arrest, expression of senescence-associated β-galactosidase activity (SA-β-gal) and senescence-associated heterochromatin foci (SAHF), and cell death were evaluated to demonstrate the reduction of senescent cells. Moreover, we have analysed colony formation capacity, and invasiveness of U2OS treated with SM and SQM.

RESULTS

Quercetin reduced the number of Doxo-induced senescent fibroblasts without affecting the proliferating one, decreasing SA-β-gal activity, cell cycle arrest markers and SAHF presence compared to not-treated senescent cells. Thus, our preliminary data show that Quercetin selectively induces cell death in senescent cells but not in the proliferating ones. Moreover, our data show that SM enhanced U2OS growth and invasiveness, while SQM reduced these aggressive behaviours.

CONCLUSIONS

Our study shows that Quercetin can exert a potential senolytic activity on Doxo-induced senescent fibroblasts, and therefore it can reduce their pro-tumoral effect on U2OS cells. Even if the mechanism by which Quercetin acts has yet to be clarified, our results demonstrate that its use to eliminate senescent fibroblasts induced as an off-target effect of Doxo could be a good strategy to reduce chemotoxicity and metastatic progression.

P030

IN VITRO EVIDENCE OF ANTIPROLIFERATIVE AND ANTIMIGRATORY EFFECTS OF METFORMIN IN MALE GERM CELL TUMORS

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BACKGROUND-AIM

Germ cell tumors (GCT) are the most common cancers in young males. In most cases, these tumors arise from the testes and rarely develop from extragonadal sites, probably as a result of primordial germ cell (PGC) migration errors. The 5-year survival of patients with GCT is high, thanks to the use of platinum-based chemotherapy, which, however, is highly toxic. Using a cell model of GCT, here we examined for the first time the antiproliferative and antimigratory effects of metformin (MET), a widely prescribed insulin-sensitizing/antidiabetic drug with emerging anticancer properties.

METHODS

SEM-1 cells, an established cell line derived from a human mediastinal GCT, were cultured in RPMI medium, supplemented with 10% FBS and antibiotics. MET (0.5-10 mM) was added 24h prior to experiments. Cell viability was studied by MTT assay and spherogenesis by seeding GCT cells in appropriate culture conditions, in the presence or absence of MET. Cell migration was evaluated by wound healing. Cyclin D1, insulin-like growth factor binding protein-1 (IGFBP1), matrix metallopeptidase-11 (MMP-11), and high-mobility group A1 (HMGA1) proteins were determined by immunoblot analysis.

RESULTS

Exposure of cells to MET (IC50 = 5 mM) resulted in a dose-dependent effect on the reduction of cell proliferation, as demonstrated by the decreased nuclear abundance of cyclin D1. Also, MET prevented SEM-1 cells to form spheroids when placed in suspension culture, and blocked SEM-1 cell migration by inhibiting IGFBP1 and MMP-11 protein expression, two factors that are involved in PGC migration during fetal life and neoplasm invasiveness. Both, IGFBP1 and MMP-11 genes are known to be under control of the transcription factor HMGA1. Consistently, in these cells, HMGA1 protein levels were markedly reduced within 12h from MET treatment.

CONCLUSIONS

Our in vitro studies show dose-dependent antiproliferative and antimigratory effects of MET in SEM-1 cells, and potentially in GCT. The anticancer properties of MET may be due to its ability to interfere with HMGA1, which is highly expressed in several cancer types and cancer cell lines, as well as in normal progenitor cells. Thus, MET may have the potential to represent a novel therapeutic agent for the treatment of GCT (i.e., alternative, or adjuvant to chemotherapy).

P031

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 establish a terminal metastatic disease, the main cause of cancer-related mortalities. CTC phenotype is characterized by the ability to survive in the bloodstream, due to the acquired resistance to detachment-induced apoptosis (anoikis). This study aims to investigate the phenotypic and metabolic reprogramming of anoikis-resistant melanoma cells, changes that acquire particular importance to target this tumor cell subpopulation. We previously found that extracellular acidosis, one hallmark of the tumor microenvironment, promotes in melanoma cells a phenotype able to survive in the bloodstream.

METHODS

A375M6 human melanoma cells grown in acidic (pH 6.7) medium for 3 months were injected into the bloodstream of immunodeficient animals and recovered through a cardiac puncture after 15 min from the injection. The heterogeneous tumor cell suspension was distributed in culture dishes and colonies were amplified and pooled together. Then, cells were exposed to a rocking condition for three following passages and, only at the end of this treatment, survived cells were studied for EMT markers and changes in the metabolic profile.

RESULTS

We found that anoikis-resistant melanoma cells express a higher level of EMT markers, such as N-Cadherin, Zeb2 and SNAIL1, associated with high invasive ability and low IKB expression, confirming that the resistance in the bloodstream needs a mesenchymal reconversion of cells. These cells were also characterized by an Oxphos metabolic reprogramming sustained by increased fatty acid and glutamine uptakes and metabolism. These findings suggest that the survival in the detachment condition is strictly related to an Oxphos metabolic profile, associated with the acquisition of an EMT phenotype.

CONCLUSIONS

Anoikis-resistant melanoma cells show a metabolic Oxphos reprogramming which could be a potential target to counteract the survival and dissemination of CTC, preventing cancer metastasis.

P032

CYTOTOXIC EFFECT OF SYNTHETIC INHIBITORS OF THE ASPARAGINE TRANSPORTERS ASCT2 AND SNAT5 IN HUMAN ACUTE LYMPHOBLASTIC LEUKAEMIA CELLS

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BACKGROUND-AIM

Acute Lymphoblastic Leukaemia (ALL) blasts express low levels of the enzyme Asparagine Synthetase (ASNS) and are thus strictly dependent on extracellular asparagine (Asn). The bacterial enzyme L-Asparaginase (ASNase) is used for ALL therapy since it depletes extracellular Asn, causing a severe nutritional stress and apoptosis of ALL blasts. Bone marrow mesenchymal stromal cells (MSCs) use extracellular glutamine to synthetize several metabolites (Taurino G et al., Mol. Met. 2022), such as Asn, which is then taken up by ALL blasts, promoting resistance to ASNase (Chiu M, Taurino G et al., Blood Adv. 2021) and ALL relapse. No information is yet available on the transport mechanisms involved in Asn uptake by ALL blasts.

METHODS

ALL cell lines were cultured in the absence or in the presence of natural or synthetic competitors: Threonine (Thr) or V9302 for ASCT2; Histidine (His) or GluγHA for SNAT5. The expression of the two transporters was lowered by gene silencing. Intracellular Asn levels were measured through LC-MS. ALL cell viability was assessed with biochemical methods and cytofluorimetry in monocultures or in co-cultures with primary MSCs derived from ALL patients.

RESULTS

In a panel of ALL lines (RS4;11, REH, NALM-6, 697), RS4;11 cells exhibit the lowest ASNS expression. In these cells, intracellular Asn fully depends on membrane transport and is significantly and additively lowered by either ASCT2 or SNAT5 silencing or inhibition. High concentrations of Thr and His hamper ALL cell proliferation, which is totally blocked in the presence of the synthetic inhibitors V9302 and GluγHA. The two inhibitors suppress mTOR activity, induce autophagy and cause nutritional stress, leading to massive ALL cell death. The cytotoxic effect is evident in both low-ASNS, ASNase-sensitive (RS4;11 and REH) and high-ASNS, ASNase-resistant (NALM-6 and 697) ALL cell lines and is not prevented by co-culture with MSCs, whose viability, however, is not affected by the inhibitors.

CONCLUSIONS

ASCT2 and SNAT5 are the transporters used by ALL blasts to internalize Asn and their inhibition causes ALL blast cytotoxicity, even in the presence of MSCs. Transporter inhibition, therefore, overcomes resistance to ASNase and may represent a novel therapeutic approach for relapsing ALL patients.

P033

SELECTIVE TARGETING OF REDOX ALTERATIONS IN MEDULLOBLASTOMA TUMORS THROUGH NANOTECHNOLOGY-BASED DELIVERY SYSTEMS

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BACKGROUND-AIM

The antidiabetic drug phenformin displays potent anticancer activity in different tumors but its mode of action is still not clear. Using medulloblastoma as a model, we have shown that at the therapeutic concentrations phenformin inhibits mitochondrial glycerol-phosphate dehydrogenase and increases intracellular NADH, activating the corepressor CtBP2 and inhibiting tumor growth. This novel biguanide-mediated redox/corepressor interplay may represent a relevant target for tumor therapy. However, the clinical use of phenformin is currently limited because of its toxicity. One way to increase its therapeutic efficacy with a reduction of its potential side effects would be the entrapment into nanocarriers able to selectively deliver the drug to medulloblastoma tissue. In this work, we have designed and prepared specific vesicular systems, called niosomes, and characterized these phenformin-loaded nanovectors in cultures of medulloblastoma cells and in animal models.

METHODS

For in vitro cytotoxicity assay, Med1-MB cells were treated with niosomes at increasing concentrations and cell viability was estimated using CellTiter-Glo Luminescent Cell Viability Assay (Promega). For cellular uptake studies, cells were treated as described above, fixed and then observed under a fluorescence microscope. In western blotting analysis cells were treated as described above, collected, lysed and the total protein was separated and visualized using enhanced chemiluminescence. For in vivo pharmacokinetic distribution, mice were treated with phenformin-loaded niosomes by tail vein injection. After 2 hours, plasma and tissue samples were collected.

RESULTS

Our results demonstrate that the use of phenformin-loaded niosomes dramatically enhance the concentration of the drug and the redox state in medulloblastoma cells, thereby improving its anti-proliferative effects. The administration of phenformin-loaded niosomes to medulloblastoma mouse models resulted in a higher accumulation of drug in the brain and an enhanced oncogenic inhibition.

CONCLUSIONS

Together, these data suggest that this novel niosome-based system could be an effective new strategy against brain cancer, to maximize the biguanide-mediated redox/corepressor interplay, and selectively target redox-dependent mediators.

P034

TRIGGERING APOPTOSIS IN MUTATED P53 CANCER CELLS: HOPS/TMUB1 AS NEW WEAPON IN CANCER THERAPY

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BACKGROUND-AIM

The p53 protein is commonly indicated as the 'guardian of the genome' due to its activities in maintaining genomic stability. Recently, the modifier HOPS/TMUB1 has been identified as a major regulator of p53, directly triggering its stabilization by inhibiting ubiquitination, and directing p53-mediated mitochondrial apoptosis in response to DNA damage. Since mutated p53 is found in more than 50% of tumors, reactivating p53 apoptotic functions in these cells may represent a breakthrough in finding new cancer therapy.

METHODS

The data presented here, using a combination of genetic, bioinformatics and biochemical approaches, showed that HOPS overexpression plays a role in balancing a defective p53-dependent system. We used human cancer cell lines expressing mutp53 forms (R273H, R248W, R175H) and Hela Hops Knock-out cells, generated using Crispr/Cas9 tech, to provide novel information on the global functioning of HOPS in mediating p53 functions.

RESULTS

We first analyzed the interaction and localization between HOPS and mutp53. Focusing mainly to cancer-related stimuli, through Co-IP analysis we assessed HOPS ability to regulate mutp53 by mitochondrial relocation, while by IF analysis we detected a reduction in p53 localization in the cytoplasm of Hops knock out cells. To gain insight into the overall functioning of HOPS, we also monitored transcriptional and non-transcriptional p53 activities to excludes mutp53 GOF activities mediated by HOPS. We finally showed that, overexpressing HOPS we were able to increase the percentage of apoptotic cancer cells, as well the release of cytochrome C, strengthening the role of HOPS in reactivating mutated p53 functionality.

CONCLUSIONS

The combination of all approaches used provide novel and stimulating evidence about the physiological significance of HOPS in controlling tumor progression by regulating all p53 forms and in triggering apoptosis as promising approach in cancer therapy. The ability to modulate the life or death of a cancer cell is recognized for its wide therapeutic potential, and HOPS, affecting both p53 WT and mutated, might shape cell choice in response to damage. Further studies are needed to investigate the biological bases underpinning the HOPS/p53 axis, and it might open a new attractive avenue in the battle against cancer.

P036

ALTERATION LEVEL OF THE MYC ACETYLATION AS AN EPIGENETIC MARKER IN THE DIAGNOSIS, PROGNOSIS AND CANCER THERAPY

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BACKGROUND-AIM

The effects of histone deacetylase inhibitors (HDACi) in advanced cancers are currently under clinical investigation. We have already demonstrated that HDACi, as SAHA, induce the death of acute myeloid leukemia (AML) cells due to the reactivation of the TRAIL gene. This selective tumor action is mediated by the MYC oncogene.
HDACi induce acetylation of MYC in lysine 323 (MYCK323ac), resulting in MYC downregulation both at transcriptional and protein level, thus unlocking expression of target genes, including TRAIL, and leading to cancer cell apoptosis.
The MYC:MAX heterodimer activates its target genes through the recruitment of the adapter protein TRRAP, which acts as a scaffold to bind complex containing histone acetyltransferase (HAT), such as Tip60 and p400. This complex leads to a state of hyperacetylation that activates the transcription of target genes.
In this work we aim to identify the HAT(s) responsible for SAHA-mediated MYC hyperacetylation easily targetable to block oncogenic activity of MYC.

METHODS

In U937, a human AML cell line, Western blot and q-RT-PCR experiments were performed to evaluate MYCK323ac, MYC, p300, GCN5 and TIP60 expression levels after SAHA and A485 treatment, alone and in combination. Immunoprecipitation experiments followed by mass spectrometry analysis were performed to evaluate the MYC interactome.

RESULTS

Western blot experiments performed in U937 cells treated with SAHA show an increase of the p300 expression level correlated with the increase in the MYCk323ac signal, suggesting a role of this HAT in the epigenetic regulation of MYC. Western blot and q-RT-PCR analyses indicate that silencing and pharmacological inhibition of p300 inhibits SAHA-mediated MYC acetylation. Immunoprecipitation experiments, followed by mass spectrometry analysis, in U937 treated with SAHA and A485, alone and in co-treatment, confirm the involvement of p300 in MYC regulation, also identifying other HAT correlates to p300, including p400, RUVBL1 and RUVBL2, which have been modulated by both SAHA and A485.

CONCLUSIONS

Our results suggest p300 and its cofactors as potential biomarkers of HDACi antitumor action, leading to blockade of MYC oncogenic activity. Further investigations are required to clarify the role of each partners in SAHA-mediated MYC regulation.

P037

ARYL HYDROCARBON RECEPTOR AGONISM ANTAGONIZES THE HYPOXIA-DRIVEN INFLAMMATION IN CYSTIC FIBROSIS

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BACKGROUND-AIM

Hypoxia contributes to the exaggerated yet ineffective airway inflammation that fails to oppose infections in cystic fibrosis (CF). However, the potential for impairment of essential immune functions by the hypoxia-inducible factor (HIF)-1α inhibition demands for a better comprehension of downstream hypoxia-dependent pathways that are amenable for manipulation.
We assessed here whether hypoxia may interfere with the activity of the aryl hydrocarbon receptor (AhR), a versatile environmental sensor highly expressed in the lungs where it plays a homeostatic role.

METHODS

We resorted to in vivo and in vitro models to study the impact of hypoxia on AhR expression and activity in human and murine CF, define the functional role of AhR in CF and assess whether AhR agonism may antagonize hypoxia-driven inflammation.

RESULTS

We demonstrated that there is an important interferential crosstalk between the AhR and HIF-1α signaling pathways in murine and human CF, in that HIF-1α induction squelched the normal AhR response through an impaired formation of the AhR: nuclear translocator heterodimer. However, functional studies and analysis of the AhR genetic variability in patients with CF proved that AhR agonism could prevent the hypoxia-driven inflammation, restore immune homeostasis and improve lung function.

CONCLUSIONS

This study emphasizes the contribution of environmental factors in CF disease progression and suggests the exploitation of the hypoxia:xenobiotic receptor cross-talk for anti-inflammatory therapy in CF.

P038

HEDGEHOG/GLI-MEK5 AXIS POSITIVELY MODULATES ERK5 ACTIVATION IN MELANOMA

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BACKGROUND-AIM

Malignant melanoma is the deadliest skin cancer, with a poor prognosis in advanced stages. We have recently reported that the Mitogen-Activated Protein kinase ERK5 is required for Hedgehog/GLI (HH/GLI)-dependent melanoma cell proliferation and that GLI1, the major downstream effector of HH/GLI signaling, positively regulates ERK5 expression by binding to a functional non-canonical GLI consensus sequence at the ERK5 promoter. We have also observed that activation of the HH/GLI pathway induces a marked increase of the upstream kinase MEK5, pointing to an additional HH/GLI-dependent mechanism that could contribute to the activation of the ERK5 pathway in melanoma.

METHODS

Melanoma cell lines used: SSM2c (wild type B-RAF), A375 (BRAFV600E) and MeWo (wild type B-RAF/NRAS, NF1 loss). Activation of the HH/GLI pathway was obtained by silencing the negative regulator PATCH1 using lentiviral vectors expressing a specific shRNA, while genetic inhibition of GLI1 was achieved using lentiviral vectors expressing GLI1-targeting shRNA.

RESULTS

Activation of the HH/GLI pathway by genetic inhibition of PTCH1 increased the expression and phosphorylation of MEK5. Chromatin Immunoprecipitation experiments performed in in SSM2c, in which the HH-GLI pathway is constitutively active, showed that genetic inhibition of GLI1 determined a reduction of RNA Polymerase Type II recruitment at and of GLI1 binding to a functional non-canonical GLI consensus sequence at MAP2K5 promoter, the gene encoding for MEK5 protein. In further support of a positive regulation of MAP2K5 by GLI1 transcription factor, GLI1 silencing resulted in the reduction of MAP2K5 mRNA in SSM2c, A375 and MeWo melanoma cell lines. Moreover, we observed that GLI2, whose mRNA is decreased upon GLI1 silencing, binds to the promoter of MAP2K5, suggesting that also GLI2 could positively contribute to MAP2K5 transcription. Finally, we found binding sites for c-MYC, a known GLI gene target, at MAP2K5 promoter, and that c-MYC binds to the promoter of MAP2K5.

CONCLUSIONS

We identified a novel HH/GLI-MEK5 axis that provides an additional mechanism through which the HH/GLI signaling regulates the activation of ERK5 in melanoma, and that could be exploited to design anticancer treatments directed to reduce MEK5/ERK5 activity.

P039

EXPLORING A DRUG REPURPOSING APPROACH TO TREAT PANCREATIC DUCTAL ADENOCARCINOMA

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BACKGROUND-AIM

The pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense tumor-associated stroma consisting of extracellular proteins, such as collagen I and fibronectin, which generate a stiff extracellular matrix. The crosstalk between tumor cells and the microenvironment promotes PDAC growth and dissemination and no effective treatment for PDAC is currently available. Recent studies showed that collagen provides a potential source of amino acids to fuel tumor growth and that PDAC cells exploit the environment to support metastatic dissemination. Pharmacological strategies to reduce the fibrotic component/collagen and the acquisition of mesenchymal traits in PDAC cells are therefore of great interest to prevent tumor growth and dissemination. Budesonide, a glucocorticoid drug commonly used to treat asthma, was recently recognized as a potent inhibitor of collagen deposition and of the acquisition of mesenchymal traits in both pluripotent stem and breast cancer cells. The aim of this study is to evaluate the potential repositioning of budesonide for treatment of PDAC.

METHODS

We have assessed the molecular and functional effects of budesonide on two independent PDAC -derived primary cell lines.

RESULTS

Immunofluorescence and WB analysis reveal that Budesonide strongly reduces collagen accumulation in vitro and in vivo, and the expression of mesenchymal markers as vimentin and fibronectin I. Accordingly, budesonide reduces PDAC cell invasion in tumor spheroids in 3D organotypic cultures, without affecting cell proliferation and apoptosis, as shown by EdU incorporation and annexinV/PI staining.
Moreover, genome-wide RNA-Seq analysis reveals that budesonide alters the expression of a large set of genes in PDAC cells, which are enriched in key biological processes including cell migration, locomotion, and collagen fibril organization.

CONCLUSIONS

Our findings that Budesonide reduces PDAC cells invasion and mesenchymal transition preventing collagen synthesis and fibrosis, suggest potential repurposing of this drug to treat PDAC progression.

P040

ALTERED TLR4 DISTRIBUTION AND DEFECTIVE LPS RESPONSIVENESS IN HUMAN PRIMARY MACROPHAGES PRE-EXPOSED TO AMORPHOUS SILICA NANOPARTICLES

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BACKGROUND-AIM

The biological effects of amorphous silica nanoparticles (ASNP) are extensively studied given their presence in biomedical products, several types of processed food and toothpastes. Increasing evidence indicates that ASNP may trigger inflammatory responses in exposed organs. Much less is known on the effects of ASNP exposure on the responses of innate immune cells to natural activators, such as PAMPs or DAMPs. We found that the pre-exposure to non-cytotoxic doses of ASNP interferes with LPS-dependent effects in human macrophage-like THP-1 cells (Bianchi et al., Nanomaterials 10, 1395, 2020). We extend here this approach to human primary monocyte-derived macrophages (MDM) and investigate the underlying mechanisms.

METHODS

Human primary monocytes were isolated from peripheral blood and differentiated to M0 MDM for 7 days in the presence of 50 ng/ml M-CSF. After 24h of pre-exposure to 10 μg/cm^2 ASNP, MDM were challenged with 1 ng/ml LPS. NFκB signalling as well as TLR4, CD14, LC3I/II and Glutamine Synthetase (GS) protein expression was investigated by western blot. Secreted cytokines were measured with ELISA, while RT-PCR was exploited to assess gene expression. TLR4 localization was assessed by immunocytochemistry in cells co-stained with LAMP1 and analysed with confocal microscopy.

RESULTS

A 24h-pre-exposure to non-cytotoxic doses of ASNP markedly inhibited the LPS-dependent induction of pro-inflammatory (TNFα, IL-6) and anti-inflammatory cytokines (IL-10). The inhibitory effect was associated with the suppression of NFκB activation, autophagy activation, intracellular sequestration of the TLR4 receptor and its partial co-localization with the lysosomal marker LAMP1. The late induction of GS by LPS was also prevented by pre-exposure to ASNP, while GS silencing did not interfere with cytokine secretion.

CONCLUSIONS

Thus, pre-exposure to ASNP leads to abnormal intracellular sequestration of TLR4, rendering macrophages less sensitive to LPS stimulation and markedly lowering the induction of genes dependent on NFκB pathway. The impairment of inflammatory response may have short-term consequences, such as a reduced efficiency of acute responses to PAMPs. However, in the long term, sub-optimal responses may also hinder resolution, thus favouring evolution towards chronicity.

P041

3D GASTRULOIDS: AN INNOVATIVE EMBRYO-LIKE STEM CELL MODEL FOR GENETIC AND PHARMACOLOGICAL SCREENING

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BACKGROUND-AIM

Gastruloids are tridimensional embryonic organoids derived from pluripotent stem cells that mimic the early stages of embryonic development and recapitulate the axial organization of the embryos. Recent studies have highlighted the potential of 3D gastruloids in different applications, and on a scale that would not be possible in living organisms, such as: i) studies of mammalian embryo development and ii) genetic and pharmacological screenings.
Here we have tested the effect on 3D gastruloids formation of a group of glucocorticoids, and in particular of budesonide. Budesonide is a glucocorticoid drug largely used to treat asthma and it has been recently identified as a potent inhibitor of the embryonic stem cell-to-mesenchymal transition (esMT)/EMT.

METHODS

By combining morphological, immunofluorescence and RNA-Seq analysis, we provide unprecedented evidence that Budesonide, but not the classical glucocorticoids (dexamethasone, fluticasone, hydrocortisone), inhibits 3D gastruloid formation by preventing exit from naive pluripotency.

RESULTS

Accordingly, Budesonide activity on 3D gastruloids is independent from the glucocorticoid receptor. Mechanistically, we propose that budesonide stabilizes cell-cell adhesions by maintaining high levels of E-caderin at the plasma membrane.

CONCLUSIONS

Our findings support the use of 3D gastruloids to identify potential teratogenic drugs, and suggest a previously unidentified adverse effect of budesonide on embryo development

P042

EXPLORING THE ANTICANCER ACTIVITY OF RUTHENIUM COMPLEXES IN MALIGNANT GLIOMA

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BACKGROUND-AIM

Glioblastoma is an aggressive and invasive brain tumour, that, despite the advances of modern therapeutic approaches, has no effective treatment. Indeed, temozolomide is the only available chemotherapeutic treatment and the median survival following diagnosis is 12 to 15 months. Ruthenium anticancer drugs have been synthesised as an alternative to the already existing platinum-based therapies. Here, we assessed the cytotoxic effects of ruthenium compounds' as well as their ability to trigger DNA damage response (DDR), to inhibit of cell proliferation, to permeabilize the lysosomal membrane and to enhance mitochondrial superoxide production.

METHODS

MTT assay was performed to investigate the drugs' cytotoxic effect of 72-hour (h) exposure on a commercial line of glioblastoma, U-87 MG. Cells were exposed to a single treatment 24 hours after seeding, or a repeated-treatment approach, one 24h and the other 72h after seeding. Cell death was assessed by Annexin V/Propidium Iodide staining and later FACS analysis. Ki67 and γH2AX were employed to assess cell proliferation and DDR. Mitochondrial superoxide was assessed employing MitoSOX. Lysosomal membrane permeabilization was assessed evaluating galectin 3 puncta.

RESULTS

Based on MTT assays, two ruthenium compounds were selected. One complex, at 0.09 μM, induced a twofold increase in cell death with a single treatment at 96h, while it reached a sixfold at 96h with a repeated-treatment approach. Proliferation steadily decreased at 72h, while DDR progressively increased in both approaches. Release of mitochondrial superoxide increased at 96h with a marked effect following the repeated treatment. The second complex at 0.8 μM exerted a mild effect with a single treatment on cell death, lysosomal permeability, and mitochondrial superoxide. However, it induced a fivefold cell death increase, a mild reduction of proliferation accompanied by a sensible effect on DDR at 72h, a marked increase in lysosomal permeability and a sharp mitochondrial superoxide production with the repeated treatment.

CONCLUSIONS

Ruthenium compounds show a pleiotropic action after cell internalization, resulting in cell cycle arrest and apoptosis.

P043

HISTIDINE-RICH GLYCOPROTEIN IN NASH-RELATED LIVER CARCINOGENESIS

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BACKGROUND-AIM

Non-Alcoholic Fatty Liver Disease (NAFLD) has recently emerged as a leading cause of chronic liver disease in the general population in Europe and in the USA acquiring clinical relevance as a large percentage of NAFLD patients can develop steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC). At present, mechanisms and mediators involved in the development of NASH-related HCC are still largely unknown. In the present study, we have investigated the pro-carcinogenic role of histidine-rich glycoprotein (HRG), a multidomain and multifunctional plasma protein abundantly produced by hepatocytes.

METHODS

The role of HRG was investigated by morphological, biochemical, cell and molecular biology approaches in: a) HRG knock-out mice (HRG-/- mice) and related wild type mice (WT mice) undergoing the NASH-related DEN/CDAA diet protocol of hepatocarcinogenesis; b) NAFLD/NASH patients carrying HCC; c) human THP1 macrophage and LX2 activated myofibroblast-like cell lines, hepatic stellate cells (HSC/MF) treated with purified HRG.

RESULTS

Following the in vivo treatment with the DEN/CDAA carcinogenic protocol, HRG-/- mice showed a significant decrease in the volume and number of NASH-related liver tumors as compared to wild-type mice. These effects were dependent on a significant reduction in the inflammatory and angiogenic response together with an increased of apoptotic rate. Moreover, HRG exerted an M1-type pro-inflammatory action on THP1 cells (by up-regulating IL-1β, TNF, IL-6 and IL-12) but also led to increased expression of the anti-inflammatory IL-10, VEGF-A and IL-23A ; c) HRG plasma levels were up-regulated in F3/F4 NASH patients as well as in those carrying HCC; d) evaluation by histological score (H-score) of liver samples from NAFDL/NASH-related HCC revealed that HRG expression was higher in peri-tumoral tissue compared to HCC nodules and correlated with a worst prognosis.

CONCLUSIONS

These results indicate that the release of HRG by hepatocytes and cancer cells has a critical role in the progression of NASH-related liver carcinogenesis.

P044

OLEOCANTHAL-ENRICHED EVOO EXTRACT FOR A COMPLEMENTARY THERAPY OF RESISTANT GASTRIC CANCER

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BACKGROUND-AIM

Gastric Cancer (GC) remains a critical problem for health system being the fifth malignancy for incidence and the fourth for mortality globally.
GC has no specific symptom, therefore the diagnosis of the majority of GC is at advanced stages when surgery, the only curative approach, is substituted or supported by chemotherapy (CT).
However frequently CT fails due to patients' resistance. The possibility to use nutraceuticals in association with CT in a "complementary therapy" to enhance efficacy of treatment and eventually limit side effects reducing dosage could represent a breakthrough in tumor therapy. The use of extra-virgin olive oil (EVOO) has gained the interest of many scientists thanks to its multiple biological activities and its extremely low toxicity for the organism. In particular, Oleocanthal (OC), an EVOO compound, characterized by an ibuprofen-like chemical structure, shows effects in many types of cancer.
The aim of this work is to verify whether an OC-enriched EVOO extract (OCF) might be useful to overcome GC resistance.

METHODS

We used the AGS gastric adenocarcinoma cell line and its resistant subpopulations selected through chronic exposure to 5-fluorouracil (5FUr), cisplatin (CISr) or paclitaxel (TAXr).
Cells treated with OCF were analyzed through MTT, Annexin V-PI cytofluorimetric assay, cell cloning ability, ROS evaluation and Western Blot.

RESULTS

We found that 60 μM OCF promotes the apoptotic death of 25-50% wild type AGS, 5FUr and TAXr, but not CISr cells, which needs at least 240 μM. We suggest that OCF efficacy may be due to cell cycle inhibition in accordance with its ability to promote ROS production, driving a p21 up-regulation mediated by p53 increase. CISr's OCF higher resistance seems to be dependent to greater levels of antioxidants enzymes counteracting OCF-induced intracellular ROS production.
Treating GC resistant cells with 60 μM OCF plus 5-fluorouracil, paclitaxel or cisplatin, we found a potentiating effect respect to mono-treatment in all resistant GC cells, including CISr.
This last finding is of particular importance in view of the therapeutic protocol represented by FLOT regimen where these drugs are used.

CONCLUSIONS

These preliminary results open-up the possibility to evaluate efficacy of OCF in vivo, as valuable adjuvant in resistant GC treatment.

P045

A MISSING ROLE OF A NEGLECTED PARASITE: LEISHMANIA INFANTUM AS A NOVEL TOOL TO PREVENT THE NLRP3-DERIVED NEUROINFLAMMATION IN MICROGLIA

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BACKGROUND-AIM

Ancient Leishmania infantum parasites exhibit impressive abilities to adapt to distinct microenvironments while suppressing killing microbicidal activities that threaten parasite persistence. In mammals, Leishmania hijacks inflammatory immune responses by macrophages (Mφ), their main cellular niche, including the activation of the NLRP3 inflammasome, a sensor complex of the innate immunity. NLRP3 is a hallmark of other immunopathologies including Alzheimer's disease (AD), where amyloid-β (Aβ) peptide accumulation is sensed by microglia (MG), the brain Mφ, resulting in an aberrant inflammatory cascade. Given that the NLRP3 is a key drug target against AD, we hypothesized that Leishmania subversion tactics might revert MG activation through NLRP3 inhibition.

METHODS

We utilized an immortalized murine microglia cell line (MMC) and ex vivo primary MG (pMG) isolated from postnatal C57BL/6 mice, infected with WT or red fluorescent L. infantum. Phagocytosis of L. infantum and Aβ was studied by live-cell imaging. Inflammatory cytokines were analyzed by ELISA and qPCR, and neurotoxic nitric oxide (NO) by the Griess assay. NLRP3 modulation was studied through caspase-1 activity, IL-1β and IL-18 release, and ASC-Speck formation. Finally, gene expression and immunofluorescence analyses served to identify the underlying mechanistic insight in Aβ-stimulated MG.

RESULTS

Our results demonstrate that L. infantum infection does not induce MG activation, thus resulting in a "silent" parasite entry. Interestingly, both L. infantum and Aβ can be concomitantly phagocytosed by both MMC and pMG, and the presence of L. infantum in Aβ-induced MG inhibits the expression of pro-inflammatory and neurotoxic IL-1β, IL-18 and NO in a dose- and parasite-dependent manner. Besides, L. infantum impairs the formation of ASC-Speck complexes and reduces caspase-1 activity by Aβ. Finally, we further found that the parasites hinder components of the NF-kB signaling and upregulate the NLRP3-negative regulator A20 upstream of the inflammasome platform.

CONCLUSIONS

Leishmania has co-evolved fascinating strategies to persist in host Mφ. Intriguingly, Leishmania infection dampens the NLRP3 inflammasome in Aβ-stimulated MG. These results may represent a potential and unprecedented bioinspired strategy against neuroinflammation in AD.

P046

THE ROLE OF PCSK9-ENRICHED EXTRACELLULAR VESICLES IN THE PATHOGENESIS OF ATHEROSCLEROSIS.

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BACKGROUND-AIM

Extracellular vesicles (EVs) mediate intercellular communication. They play a role in pathological conditions, such as atherosclerotic cardiovascular diseases (ASCVD), participating in the progression of atherosclerosis. Besides endothelial, monocytes and macrophages, vascular smooth muscle cells (SMCs) are involved in atheroma formation. Their differentiation, migration and proliferation are influenced by proprotein convertase subtilisin/kexin type 9 (PCSK9), crucial in the pathophysiology of ASCVD. Aim: To unveil the impact of PCSK9-enriched EVs on the pro-atherogenic phenotype of in vitro and in vivo models recapitulating the atherosclerotic lesion formation.

METHODS

SMCs stably overexpressing PCSK9 were generated and used along with EA.hy926 endothelial cells, THP-1 monocytes, THP-1-derived-macrophages and embryos of Danio rerio zebrafish. EVs were isolated from SMCs overexpressing or not PCSK9, respectively, EVs^{PCSK9} and EVs^{CTR}. Techniques: flow cytometry, Western blot, qPCR, nanoparticle tracking analysis, transmission electron microscopy, mitochondrial-bioenergetics analysis.

RESULTS

EVs^{CTR} and EVs^{PCSK9} expressed tetraspanins (CD9, CD63), Alix, β1-Integrin with no differences in their concentrations (625.17±235.23/mL/cell count and 926.17±815.26/mL/cell count, respectively), size (235.78±29.78 nm and 233.16±16.3 nm, respectively) and morphology. EVs^{PCSK9} carried a higher amount of PCSK9 vs EVs^{CTR}. Endothelial cells exposed to EVs^{PCSK9} showed a raised expression of adhesion molecules and pro-inflammatory markers. Concerning THP-1, exposure to EVs^{PCSK9} dramatically raised gene expression of MCP-1, IL-1α, IL-1β, IL-6 and IL-8 as well as the phosphorylation of the inflammatory protein STAT3. EVs^{PCSK9} enhanced the migratory capacity of THP-1 and decreased basal and maximal mitochondrial respiration. Inflammatory markers were raised also in THP-1-derived macrophages exposed to EVs^{PCSK9} with an increased uptake of oxLDL. Injection of EVs^{PCSK9} in the hindbrain ventricle of zebrafish embryos led to a local recruitment of neutrophil/macrophage.

CONCLUSIONS

EVs enriched in PCSK9 appear to favor a pro-atherogenic inflammatory phenotype, thus highlighting the role of PCSK9 in fueling the cyclic chronic inflammatory state of the atherosclerotic lesion.

P047

MIR-422A PROMOTES ADIPOGENESIS VIA MECP2 DOWNREGULATION IN HUMAN BONE MARROW MESENCHYMAL STROMAL

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BACKGROUND-AIM

Methyl-CpG binding protein 2 (MeCP2) is a ubiquitous transcriptional regulator. The study of this protein has been mainly focused on the central nervous system because alterations of its expression are associated with neurological disorders such as Rett syndrome. However, young patients with Rett syndrome also suffer from osteoporosis, suggesting a role of MeCP2 in the differentiation of human bone marrow mesenchymal stromal cells (hBMSCs), the precursors of osteoblasts and adipocytes. The aim of our study was to identify miRNAs able to modulate the adipogenic process and their role in MeCP2-mediated modulation of adipogenesis.

METHODS

BMSCs were induced to differentiate for 15 days with specific media. MiRNA expression was evaluated through miRNA profiling and Real-Time PCR. Differentiating cells were transfected with miRNA mimics and inhibitors. MeCP2 knockdown (KO) was achieved through MeCP2-targeting shRNA lentiviral vector. Protein expression was evaluated through Western blot analysis.

RESULTS

Here, we report a downregulation of MeCP2 in in vitro hBMSCs undergoing adipogenic differentiation (AD) and in adipocytes of human and rat bone marrow tissue samples. This modulation does not depend on MeCP2 DNA methylation nor on mRNA levels but on differentially expressed miRNAs during AD. MiR-422a and miR-483-5p are upregulated in hBMSC-derived adipocytes compared to their precursors. MiR-483-5p, but not miR-422a, is also up-regulated in hBMSC-derived osteoblasts, suggesting a specific role of the latter in the adipogenic process. Experimental modulation of miR-422a and miR-483-5p intracellular levels affected MeCP2 expression and the adipogenic process. Accordingly, KO of MeCP2 in hBMSCs increased the levels of adipogenesis-related genes. Finally, since adipocytes released a higher amount of miR-422a in culture medium compared to hBMSCs we analyzed the levels of circulating miR-422a in patients with osteoporosis – a condition characterized by increased marrow adiposity – demonstrating that are they are higher and negatively correlated with T- and Z-scores.

CONCLUSIONS

Our findings suggest that miR-422a has a role in hBMSC adipogenesis by downregulating MeCP2 and it may be used as a biomarker of bone mass loss due to the expansion of the marrow adipose tissue compartment.

P048

GENISTEIN AS A TRIGGER FACTOR OF APOPTOSIS IN CHEMORESISTANT GASTRIC CANCER CELL LINES

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BACKGROUND-AIM

Gastric cancer (GC) is one of the most widespread malignant tumors in the world: is the fifth cancer by occurrence and the fourth cause of cancer death. Since chemoresistance events are frequent, researchers often look for synthetic or natural substances which can hinder tumor progression to be used in association with chemotherapy (CT) in a "complementary therapy" enhancing efficacy of treatment and possibly limiting side effects. Among these substances is Genistein, a soy isoflavone, studied for its toxicity towards some solid tumors.
In this work we show the proapoptotic action of Genistein on chemoresistant GC cell lines and its possible usefulness to overcome chemoresistance.

METHODS

We used the ACC201 gastric adenocarcinoma cell line and its resistant subpopulations.
We first obtained the resistant cells by chronic administration of increasing doses of drugs used in clinics for GC treatment (5-Fluorouracil, Cisplatin and Paclitaxel) for several months, verifying changes in their dose-response curve through MTT assays.
After evaluating the toxicity of Genistein for both resistant and wild type (WT) cells, we chose to use 80μM Genistein in acute treatment (24H), dose that corresponds to approximately 80% of vitality in WT. First, we studied the role of Genistein in promoting cell death by AnnexinV-PI assay in flow cytometry and then we evaluated the expression of Caspase 3 and PARP by western blot.
Finally, we performed a colony formation assay (after 24H treatment with Genistein) followed by a MayGrunwald-Giemsa staining.

RESULTS

The annexin V/PI assay revealed an increase in cell death after treatment, which seems more evident in chemoresistant cells than in WT.
Western blot analyses showed an increase in cleaved Caspase 3 and PARP in treated samples. Particularly, we observed a significant increase of Caspase 3 expression in the cisplatin resistant cells.
In addition, the colony formation assay showed that Genistein could block the growth of colonies while the formation of visible colonies is observable in untreated cells.

CONCLUSIONS

These preliminary results show that Genistein promotes apoptosis in GC cells. The increase of apoptosis markers expression in resistant cells suggests that Genistein is more efficient against resistant cells and could have a role as additive agent in therapy.

P049

A GENE EDITING APPROACH REVEALS THAT MYC IRES SEQUENCE DOES NOT MEDIATE CAP-INDEPENDENT TRANSLATION AND RESISTANCE TO STRESS CONDITIONS

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BACKGROUND-AIM

The Internal Ribosome Entry Site (IRES) is a region located in viral transcripts to allow the translation of viral proteins when cellular cap-dependent protein synthesis is inhibited by the infection. While in the viral context the existence of IRESes is widely accepted, the possibility that mammalian mRNA could undergo IRES mediated translation is still debated. A well characterized mammalian IRES resides in the 5'UTR of the proto-oncogene MYC. It was hypothesized that, when the cell is subjected to stress conditions, general protein synthesis is inhibited and, to escape death, cells activate IRES-mediated translation of key mRNA, to maintain or even increase their translation. In this view, IRES mediated translation of some transcripts, including MYC, could also be exploited by cancer cells to survive to the unfavorable, stressing conditions occurring during cancer development, such as metabolic, replication or ER stresses. However, many authors argue against the experimental approach used to identify mammalian IRESes,
especially the widely used bicistronic reporter assay.
Aim of the present study was to characterize MYC IRES function using a reliable and physiological approach of genome editing, to give a conclusive answer to this relevant question.

METHODS

To address the role of MYC IRES, we generated a cell line using the CRISPR-Cas9 technique to remove the region previously identified as the IRES sequence. The cells obtained (ΔIRES) were subjected to stress conditions to evaluate MYC levels and cell viability.

RESULTS

We first tested the ability of the IRES region to mediate the cap-independent translation of MYC. We used in vitro transcription of a reporter mRNA fused with the IRES with or without cap, lentiviral mediated knockdown of the cap-binding factor eIF4E and interference of mTOR. In all cases tested, MYC protein synthesis was abolished to the same extent in ΔIRES and control cells, regardless the presence of the IRES region. We then exposed ΔIRES cells to known inductors of cellular stress to test their ability to survive and maintain MYC expression. We tested genotoxic, metabolic, oxidative and ER stress and in all cases ΔIRES were as sensitive as control cells to the stress conditions and expressed MYC at equal levels.

CONCLUSIONS

Overall, we used a more physiological approach to demonstrate that the region previously identified as MYC IRES is not able to mediate MYC cap-independent translation and is not involved in the cellular response to stress conditions.

P050

ADVANCED THERAPEUTIC MEDICINAL PRODUCTS FOR HYBRIDOMA-BASED TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER

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BACKGROUND-AIM

Breast cancer is the most frequent cancer in the female population and is the leading cause of cancer-associated mortality. Indeed, about 17% of all cancer-related deaths are due to this carcinoma. Currently, therapy for this type of tumor is mainly based on cytotoxic chemotherapy, but cancer immunotherapy could represent a concrete alternative to the latter. The aim of the 'Immuno-Cluster' study is to verify the correspondence of the end product obtained by means of an open system and the one obtained with a bioreactor, and to develop a safe and effective clinical protocol for the treatment of triple-negative breast cancer based on immunotherapy with an autologous vaccine. To date, this therapy is extremely expensive, which is why the possibility of exploiting a bioreactor could significantly reduce production costs. Hence, we characterized and compared buffy coat derived immature dendritic cells (iDCs) obtained with an open system (class I laminar flow hood) and a closed system (bioreactor).

METHODS

14 samples obtained with both methods (open system and closed system) have been analyzed for the expression levels of CD14 and CD209 and for the endocytic capacity of this cell population (FITC-Dextran) by means of flow cytometry analysis to evaluate the correct differentiation of iDCs.

RESULTS

Our data demonstrated an enrichment in CD209+/CD14- iDCs population in the the samples analyzed, and an enhanced endocytic capacity calculated as the percentage of FITC-Dextran intake.

CONCLUSIONS

Final results showed that the iDCs population obtained with the closed system is comparable to the one obtained with the classical open system, therefore the production of the cellular drug treatment in a bioreactor that ensures sterile conditions for the sample throughout the process, could lead to a reduction in production costs, making this type of therapy compatible with the NHS budget and thus available to all patients who could benefit from it.

P051

IMPACT OF THE COVID-19 PANDEMIC ON THE NUMBER OF CASES OF BLOODSTREAM INFECTIONS AND RELATED RESISTANCES AT THE AZIENDA SANITARIA UNIVERSITARIA FRIULI CENTRALE (ASUFC).

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BACKGROUND-AIM

Blood cultures (BCx) represent the gold standard in diagnosis and management of bloodstream infections, estimated in about 20 million cases/year with a mortality of 26%; the agents mainly involved are bacteria. The COVID-19 pandemic emergency resulted in an increase in frail patients (pts) admitted to hospital, predisposed to secondary infections.
The aim of the study is to evaluate the impact of the COVID-19 pandemic on the number (No.) of bacteremia and the related resistance mechanisms (RES) in ASUFC.

METHODS

Through the "Mercury" program in use in our Hospital, we collected BCx data of the samples analyzed by Microbiology Department of ASUFC and tested on BD (Becton, Dickinson and company) BACTEC FX instrument, comparing the prepandemic (March-December 2019) and pandemic (March-December 2020) periods.
We used the Python development environment JupyterLab (version 3.2.1) of Anaconda distribution and Prism 9 for MacOS from GraphPad Software, LLC, for data and graphs analytics.

RESULTS

In 2019 the No. of positive (POS) BCx was 5768 (out of 48398 tests) and the No. of isolated pathogenic bacteria in BCx was 948, with RES reported 112, while in 2020 the No. of POS BCx was 4344 (out of 42188 tests) and the No. of isolated pathogenic bacteria in BCx was 543, with RES reported 133.

CONCLUSIONS

Although the results show a statistically significant decline of 12.8% in the No. of BCx analyzed between 2019 and 2020 (Fisher's exact test, $p<0.0001$), there was no difference in the incidence of POS BCx tests between the two periods studied (10.3% out of total BCx tested in 2019 versus 11.9% in 2020).
In 2020 there was a statistically significant decrease in the No. of isolated bacteria of 42.7% (Fisher's exact test, $p<0.0001$) and a simultaneous statistically significant increase in bacteria producing RES by 18.75% (Fisher's exact test, $p <0.0001$).
At last there were not statistically significant differences in the distributions of isolated pathogenic bacteria (Chi-Square test, p 0.9567) and in the RES microorganisms (Chi-Square test, p 0.5736) between the two periods evaluated: in both cases the most frequently isolated was Escherichia coli expressing Extended Spectrum Beta-Lactamase RES (ESBL).
In our experience the data collected suggest that in ASUFC the COVID-19 pandemic did not impact on the No. of bacteremia, but may have contributed to select and increase the number of pathogens expressing RES.

P052

BIOLOGICAL CONDITIONS RELATED TO FRAILTY INFLUENCE THE BEHAVIOR OF RENAL STEM/PROGENITOR CELLS GROWN AS NEPHROSPHERES

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BACKGROUND-AIM

Frailty is an age-related syndrome characterized by a reduced resilience to external stressors. This syndrome predisposes to the development of disability and other adverse outcomes. Currently, the most convincing mechanism explaining frailty pathophysiology is the state of mild chronic inflammation and the related increased oxidative stress. Different studies evidenced an association between frailty and kidney dysfunction. Therefore, the aim of this study is to evaluate biological conditions related to frailty (oxidative, inflammatory status and DNA damage) and if they can modulate the renal stem cells (RSC) behavior.

METHODS

Blood from frail, non-frail and young subjects was collected and plasma was separated for the analysis of 40 cytokines by Luminex, of the amount of Oxysterols by isotope dilution mass spectrometry and of 8-Hydroxy-2-deoxy guanosine (8-OH-dG) by ELISA. DNA damage was assessed by analyzing the #H2AX+ circulating hematopoietic progenitor stem cells (cHPSC) by FACS. Nephrosphere (NS) cultures, enriched in RSC, were established from normal human kidney tissues, and treated with 10% plasma of the enrolled subjects. Self-renewal, viability, proliferation, and DNA damage were assessed.

RESULTS

The analysis of frail plasma, compared to non-frail and young, showed a significant increase of oxysterols and of 8-OH-dG, products of cholesterol and nucleic acid oxidation, and a characteristic profile of inflammatory cytokines. cHPSC from frail subjects presented a higher percentage of #H2AX+ cells compared to non-frail and young. NS cells treated with plasma of frail subjects showed a significant decrease of self-renewal ability with significantly higher intracellular ROS and percentage of cells positive for DNA damage.

Correlation and integration of all the data provided a biological and molecular panel of frailty, identifying a different qualitative phenotype in frails compared with others. PCA permitted to classify all the samples in the three categories without knowing the clinical classification.

CONCLUSIONS

Factors present in the frail plasma could alter NS cell characteristics, raising the hypothesis of a possible role of biological mediators related to frailty in the modulation of stem/progenitor cell behavior.

P053

THE PROGNOSTIC SIGNIFICANCE OF CIRCULATING MYELOID-DERIVED SUPPRESSOR CELLS IN NON-SMALL CELL LUNG CANCER: A LITERATURE-BASED META-ANALYSIS.

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BACKGROUND-AIM

Immunotherapy is the main standard treatment for non-small cell lung cancer (NSCLC) patients. Immune suppressive cells in tumor microenvironment can counteract its efficacy. Myeloid-derived suppressor cells (MDSCs) include two major subsets: polymorphonuclear (PMN-MDSCs) and monocytic (M-MDSCs). Many studies explored the prognostic impact of these cell populations in NSCLC patients.

METHODS

We selected studies for a meta-analysis, which compares prognosis between patients with high vs low circulating MDSC levels. We collected hazard ratios (HRs) and relative 95% confidence intervals (CIs) in terms of progression-free survival (PFS) or recurrence-free survival (RFS), and overall survival (OS).

RESULTS

Among 139 studies retrieved from literature search, 14 eligible studies (905 NSCLC patients) met inclusion criteria. Low circulating MDSC levels favor a better PFS/RFS (HR = 1.84; 95% CI = 1.28-2.65), and OS (HR = 1.78; 95% CI = 1.29-2.46). The subgroup analysis based on MDSC subtypes (total-, PMN-, and M-MDSCs) obtained a statistical significance only for M-MDSCs, both in terms of PFS/RFS (HR = 2.67; 95% CI = 2.04-3.50), and OS (HR = 2.10; 95% CI = 1.61-2.75).

CONCLUSIONS

NSCLC patients bearing high M-MDSC levels in peripheral blood experience a worse prognosis than those with low levels, both in terms of PFS/RFS and OS. This finding suggests that detecting and targeting this MDSC subset could help to improve NSCLC treatment efficacy.

P054

ONCOSTATIN M IS OVEREXPRESSED IN NASH-RELATED HEPATOCELLULAR CARCINOMA AND PROMOTES A PRO-TUMORIGENIC INFLAMMATORY RESPONSE

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BACKGROUND-AIM

Oncostatin M (OSM) is a pleiotropic cytokine belonging to the interleukin (IL)-6 family that has been proposed to contribute to theprogression of chronic liver diseases, hepatocellular carcinoma (HCC) development and metastasis. High levels of OSM was observed in cirrhotic patients with different etiology carrying HCC. Here we investigated the role of OSM in relation to the development of HCC in non-alcoholic steatohepatitis (NASH) background.

METHODS

By employing morphological, biochemical, cellular and molecular biology techniques we investigated the role of OSM in NASH-related HCC taking advantage of: a) a cohort of NASH patients with or without HCC; b) a mouse model of NASH-related liver carcinogenesis (DEN/CDAA protocol); c) human macrophage cell lines exposed to human recombinant OSM (hrOSM) in vitro.

RESULTS

Serum OSM levels are significantly higher in patients carrying NASH-related HCC, as compared to those with viral aetiologies and their increase are paralleled the disease progression from simple steatosis to the development of HCC. Noteworthy, OSM serum levels are significantly higher in patients with intermediate/advanced HCC and correlated with poor survival. In human liver biopsies obtained from patients with NASH-related HCC, OSM is expressed in cancer cells in relation to the staging of HCC as well as in macrophages infiltrating tumour. Accordingly, OSM expression is increased in murine NASH-related liver tumours and correlated with F4/80 gene expression, suggesting an interplay between OSM and macrophages recruitment/functions in the tumor microenvironment. In particular, OSM transcript levels is correlated with M1 macrophage polarization markers and, more significantly, with M2 markers. Of relevance, OSM expression is correlated with new identified NASH-associated macrophages (NAM) markers, TREM-2 and CD-9. The ability of OSM to promote M2 polarization, observed in human THP1 macrophages exposed to hrOSM, is due to activation of STAT3 and PI-3K/Akt signaling pathways.

CONCLUSIONS

Patients with HCC arising on a NASH background showed increased OSM serum levels that correlate with clinical parameters and disease outcome. Experimental data highlight a pro-carcinogenic contribution for OSM in NASH, by promoting pro-tumorigenic inflammation and eventually modulating tumor escape.

P055

CIRCULATING FORMS OF PLASMA TRANSTHYRETIN IN PATIENTS WITH WILD-TYPE TRANSTHYRETIN AMYLOIDOSIS AND EFFECTS OF TREATMENT WITH TAFAMIDIS

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BACKGROUND-AIM

Transthyretin (TTR) is a homotetrameric 55-KDa plasma protein that transports thyroxin and retinol complexed to retinol-binding protein (RBP). TTR misfolding and aggregation can lead to the extracellular deposition of amyloid (ATTR) representing one of the most frequent form of amyloidosis in elderly. Aim of this study is to develop a native electrophoretic method to characterize circulating TTR in plasma samples of ATTR patients before and during treatment with tafamidis, a TTR stabilizer.

METHODS

Plasma from ATTR patients (n=6), collected before (T0) and during tafamidis treatment, and plasma of healthy controls (n=6) were obtained from Fondazione Toscana G. Monasterio (Pisa, Italy). Plasma samples were separated on a native 4–20% Tris-Gly polyacrylamide gel. Western blot analysis was performed with anti-TTR (DAKO) or anti-RBP (Siemens Healthineer) antibodies. Proteins were detected by Clarity ECL substrate (BioRad).

RESULTS

Circulating forms of TTR were qualitatively similar between ATTRwt patients at T0 and controls. In both groups, the most represented forms were: TTR dimers or trimers (~37-50 kDa), TTR tetramers complexed with RBP protein in 1:1 ratio (~80 kDa,) or 1:2 ratio (~100 kDa), and high molecular weight (MW) aggregates (>150 kDa). Neither TTR monomers nor the tetramers were visible. RBP protein was detectable in association with TTR tetramers and some of the higher MW fractions (~150 kDa, >250 kDa). Following tafamidis treatment, all ATTRwt patients displayed a progressive increase of the intensity of the band corresponding to TTR-RBP complexes, in agreement with the drug stabilizing action on TTR tetramers. Interestingly dimers and trimers, detectable at T0, were progressively lost during tafamidis treatment.

CONCLUSIONS

The native electrophoretic allowed us to detect several plasma circulating TTR fractions. Data suggest a similar pattern of circulating TTR both in patients and healthy control: TTR tetramer exists only complexed with RBP and in equilibrium with low and high MW forms. Furthermore, the method allowed to appreciate the stabilizing effect of Tafamidis on circulating TTR tetramers complexed with RBP. The study of circulating TTR fractions can expand our knowledge on mechanisms triggering its destabilization even when not mutated.

P056

EFFECT OF ORTHOSILICIC ACID AND VITAMIN K2 IN MESENCHYMAL STEM CELLS OSTEOGENIC DIFFERENTIATION IN VITRO: POTENTIAL ROLE IN OSTEOPOROSIS PREVENTION AND TREATMENT.

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BACKGROUND-AIM

The increasing world's older population has led to a particular interest in Age Related Diseases, such as osteoporosis. Traditional medicine has given way to a new field of study, that involves nutraceuticals. Unlike conventional osteoporosis drug treatments, most of natural compounds show no side effects. Several independent studies have demonstrated how Orthosilicic acid (OA) and Vitamin K2 (VK2) can induce osteoblastogenesis, through the expression of osteogenic mediators. However, the combination of the two has not been tested yet. In this study, we aimed to assess the osteogenic activity of BlastiMin Complex™ components (Mivell, Italy) – OA and VK2 – and the synergistic effect of their combination in vitro.

METHODS

Human Bone Marrow derived Mesenchymal Stromal Cells (hBM-MSCs) have been used to test osteogenic activity of BlastiMin Complex™. Cell viability has been determined with MTT assay and major osteogenesis markers modulation have been investigated by mRNA and protein expression analysis, respectively via qRT-PCR and Western Blotting.

RESULTS

We firstly evaluated dose- and time-dependent cytotoxicity of OA and VK2. The concentrations to be used for subsequent experiments were selected based on cell viability > 70% compared to that of control and resulted to be 75 μM OA and 0,1 μM VK2, whether used individually or in combination (MIX). After 7 days' treatment the MIX significantly increased mRNA expression of early osteogenic marker RUNX2 and late osteogenic marker OCN, with a synergistic effect for RUNX2, whereas after 14 days' treatment Col1a1 and OCN expression increased significantly and synergistically. These data were confirmed by western blot analyses.

CONCLUSIONS

These results show that OA and VK2 improve osteogenesis in vitro, especially when combined, thus suggesting the effectiveness and safety of their use in prevention of osteoporosis and as adjuvants in treatment of osteopenia. This hypothesis will be tested in the near future: the study on osteopenic subjects was approved by the ethical committee of Regione Marche and we have just started recruitment.

P057

MILD EXERCISE MODULATES THE PROTEOMIC PROFILE OF THE AGING GASTROCNEMIUS MUSCLE.

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BACKGROUND-AIM

During aging, skeletal muscles undergo a progressive mass loss and strength decline which may also lead to sarcopenia. Several studies demonstrated that physical exercise performed throughout life can mitigate the consequences of aging and delay the occurrence and progression of sarcopenia. However, it is unclear if the beneficial effects of physical exercise, even if started in adulthood, can increase the efficiency and the strength of skeletal muscles.

METHODS

Old mice (22 months) underwent 12 weeks of running on a treadmill (30 min/ day at a speed of 9 m/min for 3 days a week) or were kept in their cage under a sedentary lifestyle condition. Proteins from the gastrocnemius muscle were obtained using three sequential extractions (suitable for hydrophilic, hydrophobic, and crosslinked proteins), and each preparation was analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Raw data were analyzed using MaxQuant software and the implemented Andromeda software.

RESULTS

Of the 1505 proteins that were identified, a total of 197 proteins were significantly modified by physical exercise. Bioinformatic analyses by Metascape, allowing to evaluate protein-protein interactions, revealed that differentially expressed proteins were grouped into five subnetworks comprised of ribosome system, mitochondria and electron transport chain, muscle contraction, calcium regulation, collagen biosynthesis and modifying enzymes.

CONCLUSIONS

Data show that i) aging skeletal muscle can still respond to mild physical exercise, ii) the mitochondrial compartment is activated to cope the increased energy demand, iii) depletion of components of the translation machinery may reduce the risk to accumulate damaged proteins and can reduce energy consuming processes, thus increasing energy availability and longevity (as demonstrated in several model organisms), iv) collagen downregulation can decrease the extracellular matrix stiffness thus promoting both detection and response to mechanical forces which can be converted into signaling pathways that modulate cell shape, metabolic status and calcium storage and release.

P058

PROGNOSTIC VALUE OF SOLUBLE ST2, HIGH-SENSITIVITY CARDIAC TROPONIN, AND NT-PROBNP IN TYPE 2 DIABETES: A 15-YEAR RETROSPECTIVE STUDY

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BACKGROUND-AIM

Patients with type 2 diabetes mellitus (T2DM) present an increased risk of cardiovascular (CV) disease and excess CV-related mortality. Beyond the established role of brain natriuretic peptide (BNP) and cardiac troponins (cTn), other non-cardiac-specific biomarkers are emerging as predictors of CV outcomes in T2DM. Here we aimed at investigating the association of high-sensitivity (hs)-cTnI, N-terminal (NT)-proBNP, and sST2, alone or in combination, with all-cause mortality and development of diabetic complications, including MACE, in patients with T2DM. We compared the levels of these biomarkers between patients with T2DM and healthy control subjects and assessing their correlations with variables assessing blood glucose control, organ damage, and T2DM clinical features.

METHODS

Serum levels of soluble suppressor-of-tumorigenesis 2 (sST2), high-sensitivity (hs)-cTnI, and N-terminal (NT)-proBNP were assessed in 568 patients with T2DM and 115 healthy control subjects (CTR) through automated immunoassays. Their association with all-cause mortality and development of diabetic complications was tested in T2DM patients over a median follow-up period of 202 months using Cox models and logistic regressions.

RESULTS

Serum sST2 followed an increasing trend from CTR to uncomplicated T2DM patients (T2DM-NC) to patients with at least one complication (T2DM-C), while hs-cTnI was significantly higher in T2DM-C compared to CTR but not to T2DM-NC. A graded association was found between sST2 (HR, 2.76 [95%CI , 1.20-6.33] for ≥32.0 ng/mL and 2.00 [1.02-3.94] for 16.5-32.0 ng/mL compared to <16.5 ng/mL), NT-proBNP (HR, 2.04 [1.90-4.55] for ≥337 ng/L and 1.48 [1.05-2.10] for 89-337 ng/L compared to <89 ng/L,), and 15-year mortality in T2DM patients. Hs-cTnI ≥7.8 ng/L was associated with an increased risk of mortality (HR, 1.63 [1.02-2.62]). A combined 'cardiac score' based on sST2, hs-cTnI, and NT-proBNP was significantly associated with the all-cause mortality risk (HR, 1.35 [1.19-1.53], C-statistic=0.739) and development of MACE.

CONCLUSIONS

sST2, hs-cTnI, and NT-proBNP are associated with 15-year mortality and onset of MACE in T2DM. The long-term prognostic value of sST2 and its ability to track variables related to insulin resistance and associated metabolic disorders support its implementation into routine clinical practice.

P059

LIVE VERSUS FIXED CELL BASED ASSAYS FOR ANTI-MOG ANTIBODIES DETECTION IN SERUM AND CEREBRO-SPINAL FLUID.

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BACKGROUND-AIM

Myelin oligodendrocyte glycoprotein antibody disorder (MOGAD) is a demyelinating disease of the central nervous system that affects in both adults and children. Even though there are clinical phenotypic overlaps with neuroinflammatory disorders, the presence of anti-MOG in serum and cerebro-spinal fluid (CSF) allow the diagnosis of MOGAD. The cell-based assay (CBA) uses cell lines transfected with plasmids encoding the human MOG sequence and is extensively used for the detection of anti-MOG antibodies. Although the commercial CBA on fixed cells (CBA-IF) have demonstrated good performances, several studies have documented a better sensitivity and specificity of CBA on live cells (CBA-FC). In this study we compared the performance of commercial CBA-IF with the home-made CBA-FC.

METHODS

We performed commercial CBA-IF on epithelial HEK293 cells analyzed by indirect immunofluorescence (Euroimmun, Germany) and home-made CBA-FC on glioblastoma cells evaluated by flow cytometry. Tests were conducted on sera of patients with suspected MOGAD (n=94, M:42/F:52, age 42±20 yrs) that referred to Hospital of Udine. Only in 17 cases (18.1%), cerebrospinal fluid (CSF) samples were also tested. Cytofluorimetric results were evaluated as ratio of mean fluorescence intensity between MOG-expressing and non-transfected cells, and a threshold obtained from a pool of control sera, tested negative for anti-MOG antibodies, was used to establish the cut-off value in every analytical session.

RESULTS

By comparing the two CBAs, it can be seen that 8/94 samples resulted positive and 72/94 samples resulted negative simultaneously with both methods (85.1% of agreement, Cohen's kappa=0.45). Among those samples that tested positive with CBA-FC (19/94), only 8/94 resulted positive also with CBA-IF (two at high-titer and six at low-titer). With regard to CSF samples, the agreement was substantial (94.1%; Cohen's kappa=0.63).

CONCLUSIONS

Our findings show a good agreement between the two methods on CSF, while moderate agreement is shown in the serum experiments. Discordant data could be attributed to the better expression of MOG conformational epitopes in live neuronal cells than in fixed epithelial cells. Therefore, CBA-FC ensured a clearer identification even of patients classified low-titer positive by CBA-IF.

P060

25(OH)VITAMIN D AND PSA SERUM LEVELS: IS THERE A LINK?

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BACKGROUND-AIM

Prostate cancer (PC) is the most common non-cutaneous malignancy in men worldwide and it represents the fifth cause of death. It has long been recognized that dietary habits can notably impact prostate health and improve the benefit of traditional medical care. Diet is thought an integral part of different prostatic disease, and among the food components one considered very relevant is vitamin D. Recent studies hypothesized that vitamin D supplementation reduces circulating androgen levels and PSA secretion, inhibits cell growth of the hormone-sensitive PC cell line, inhibits neoangiogenesis and improves apoptosis. However, the results are conflicting and inconsistent. Furthermore, the use of vitamin D as a therapeutic addition to PC treatments has not achieved consistently positive results to date.

METHODS

Our group conducted a study in a city of Central Italy, carrying out laboratory test of PSA and 25(OH)vitamin D concentrations on a cohort of one hundred patients who had joined a PC screening campaign to verify what is widely hypothesized in the literature, that is the existence of a correlation between the levels of PSA and those of 25(OH)vitamin D. The same patients underwent a urological examination immediately after the blood collection, and on that occasion, we administered a questionnaire which was then used only for the descriptive analysis of the sample, obtaining information on family history, on pathological and pharmacological anamnesis and on lifestyle in terms of sport practiced and eating habits. Based on the results of the blood tests, patients were stratified into two groups for PSA (physiological <4 ng/ml _ pathological> 4ng/ml) and three groups for 25(OH)vitamin D (0-20,9 ng/ml deficiency _ 21-30 ng/ml insufficiency _ 30-100 ng/ml recommendable levels).

RESULTS

By processing the data with the STATA biostatistics program and calculating the P-value on the Pearson chi-square test (0.279), no clear correlation was found between serum 25(OH)vitamin D concentration levels and elevated PSA values.

CONCLUSIONS

Further research or ecological studies are needed in a wider geographical area with particular attention to Vitamin D supplementation, immunomarkers and other indicators of health to confirm the absence of correlation, verified in our study. Other possible different hypotheses to be evaluated are represented by the role of calcium intake as a confounding factor in the Vitamin D / PC association and the role of solar radiation in the metabolism of Vitamin D.

P061

AT THE CROSSROAD OF CANCER-ESCAPE: HOPS/TMUB1 INVOLVEMENT IN CELLULAR SENESCENCE

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BACKGROUND-AIM

The ubiquitin-like modifier HOPS/TMUB1 has been described to drive p53-dependent apoptosis, by controlling p53 stability and localization. As well as apoptosis, cellular senescence has been assumed to be a potent anticancer mechanism. Since HOPS has been reported to control p19Arf half-life and localization and given the role of p19Arf as senescence marker, here we will sustain HOPS engagement in senescence by OIS.

METHODS

Over- (HOPShigh) and down-expressing (HOPSlow) HOPS stable MEFp53-/- cells were exposed to genotoxic stress upon RasV12G transfection. The proliferating phenotype was tested by growth curve, crystal violet colony assay and soft agar assay. Senescent phenotype was assessed by β-galactosidase assay. RasV12G stable MEFp53-/- clones were used for xenograft assays in NOD/SCID mice. Development of masses was recorded. DNA microarrays were performed in HOPShigh vs HOPSlow vs controls.

RESULTS

RasV12G-dependent genotoxic stress induced an increase in HOPShigh cells for both p16 and p19Arf senescence markers, suggesting a role in OIS modulation. The measure of β-galactosidase activity for senescent outcome showed almost absent response to OIS in HOPSlow cells, while positive cells increased in HOPShigh cells. The enhancement of growth rate, loss of contact inhibition and attachment independent growth, in HOPSlow vs HOPShigh and controls revealed an increased proliferative potential. The xenograft assays showed the shortening of development time in HOPSlow engrafted mice vs control groups, while the masses in HOPShigh engrafted mice developed later or didn’t appear at all. In general, we recorded a reduced number of masses bearing mice in HOPShigh engrafted group vs both HOPSlow and controls. By the measure of the size, we observed a volume reduction of a 1 to 16 ratio in HOPShigh vs HOPSlow masses. The DNA microarrays on HOPShigh, HOPSlow and HOPSwt MEFp53-/- revealed a HOPS-dependent modulation of genes involved in the onset of senescence and SASP.

CONCLUSIONS

Our study indicates HOPS as a regulator of senescence as cancer escape mechanism. In concert with previous investigations, this report depicts HOPS to be at the crossroad in control of senescence and apoptosis outcome in response to genotoxic stress. Here we candidate HOPS as oncosuppressor acting in the subtle balancing between proliferation and cell arrest by managing both apoptosis and senescence activation. Ultimately, these data suggest the use of HOPS as possible novel therapeutic tool in oncology.

P062

GOLD NANOPARTICLES AND ENDOTHELIAL PROGENITOR CELLS: A WIN-WIN ALLIANCE FOR TARGETING TUMORS

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BACKGROUND-AIM

Plasmonic photothermal therapy utilizes biologically inert Near Infrared (NIR) gold nanoparticles (AuNPs) that convert light into heat capable of eliminating cancerous tissue. This approach has lower morbidity than surgical resection and can potentially synergize with other treatment modalities including chemotherapy and immunotherapy. In this work, we propose tumor tropic cellular vectors, called Endothelial Colony Forming Cells (ECFCs), enriched with gold chitosan-coated nanorods (AuNRs). ECFCs display a great capability to intake AuNRs without losing viability and exerting an in vitro and in vivo antitumor activity per se.

METHODS

Conventional optical and Transmission electron microscopes (TEM), Photoacoustic imaging (PA) were used to evaluate AUNRs intracellular uptake in Melanoma cells (M6) and ECFCs. Melanoma spheroids were employed to investigate the behavior of AuNRs-ECFC in 3D-culture. The tumor tropic properties of AuNRs-ECFC were confirmed in vivo, using a human melanoma xenograft rat model.

RESULTS

The PA signal provided from ECFC loaded with AuNRs exhibited a stronger enhancement compared to AuNRs-M6. As expected, ECFCs loaded with AuNRs, thanks to their ability to enter the spheroid, exert their antitumor activity by reducing the volume of the sphere, compared to control spheroids plated with unloaded ECFCs. Besides, the PA signal provided from AuNR–ECFCs inside spheroids exhibited a strong enhancement compared to M6-AuNRs ones. Histological analyses of explanted tumor mass demonstrate that gold is still retained after 1 week from injection and organs including liver, spleen, kidney, and lung did not show any morphological alteration compared to control rats treated with unloaded ECFCs.

CONCLUSIONS

We demonstrated in vitro that AuNRs-loaded ECFCs are able to generate higher photoacoustic signals than AuNRs loaded in M6 cells. 3D cultures confirm the cytostatic effect of AuNRs-ECFC on tumor. In vivo, we show, via immunohistochemical analysis, a great tumor-homing efficiency of AuNRs–ECFCs after a bolus intravenous administration and their permanence inside the tumor masses 1 week after administration.

P063

KIR AND COVID-19. A PILOT STUDY CONDUCTED ON SICILIAN PATIENTS.

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BACKGROUND-AIM

Killer immunoglobulin-like receptors (KIRs) regulate the activation of natural killer cells through their interaction with human leukocyte antigens (HLA) class I. KIR and HLA loci are highly polymorphic and certain KIR-HLA combinations have been found to protect against viral infections. In this study, we tested our hypothesis that the KIR/HLA repertoire also influences the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the pathophysiology of the relative coronavirus disease-2019 (COVID-19).

METHODS

We typed KIR and HLA genes in 45 symptomatic patients hospitalized for COVID-19 (with evidence of lower respiratory disease during clinical assessment or imaging) and 45 asymptomatic patients (diagnosed by real-time RT-PCR with no symptoms consistent with COVID-19). Using the PCR-specific primer technique, the DNA of cases and controls was genotyped for the presence of the three major KIR ligand groups (HLA-C1, HLA-C2 and HLA-Bw4, both HLA-B and HLA-A loci) and for both inhibitory and activating KIRs. KIR gene profiles were determined by the presence or absence of each KIR gene. The distribution of each KIR genotype, KIR gene, HLA ligand, and KIR-HLA combination between the study groups was estimated by chi-square.

RESULTS

No significant variation in the frequency of both HLA allele and KIR gene between the symptomatic patients and asymptomatic ones was observed.

CONCLUSIONS

The pandemic caused by SARS-CoV-2 led to the death of millions of people worldwide and thousands more infected individuals developed sequelae due to COVID-19. The development of several studies has contributed to the knowledge about the evolution of SARS-CoV-2 infection and the disease to more severe forms. Some of them have shown the role of the KIR/HLA combination and of some specific alleles in the pathogenesis and in the severity of COVID-19. From our data, we have not found any significant association between KIR/HLA genotype and COVID-19 outcome. It would be desirable to expand our cohort groups by considering the severe COVID-19 form represented by Intensive Care Unit (ICU) patients.

P064

HUMORAL AND T-CELL RESPONSE TO COVID-19 VACCINE BOOSTER IN PATIENTS WITH AUTOIMMUNE SYSTEMIC DISEASES.

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BACKGROUND-AIM

Successful vaccination is key to avoiding severe COVID-19 complications, especially in frail patients such as people with autoimmune systemic diseases (ASDs). However, information regarding humoral and cellular response to COVID-19 vaccine booster in this setting is limited.

We aimed at assessing the COVID-19 vaccine booster immunogenicity (neutralizing IgGs and IFN-γ levels) in 17 ASD patients, compared to 17 healthy controls (HCs).

METHODS

Immunogenicity of COVID-19 vaccine was evaluated, 3 weeks after the booster, in 17 ASD subjects (12 (71%) females, mean age 68.8±15.3 yrs) and in 17 age and sex matched HCs. Neutralizing IgGs against SARS-CoV-2 were measured by Quant antibody (Abbott) and expressed as Binding Antibody Units (BAU)/mL (positive test ≥7 BAU/mL, sub-optimal test ≤70 BAU/mL). Cellular response was assessed by ELISA, measuring IFN-γ levels after whole blood stimulation with SARS-CoV-2 antigens (QuantiFERON, positive test ≥0.15 IU/mL).

RESULTS

One patient (female, 60 yrs) that reported a pre-booster COVID-19 only when the experimental procedure was concluded, was excluded from analysis. Humoral response was significantly lower in ASDs compared to HCs (1909±690.2 vs 10488±454.3 BAU/mL, p<0.0001); 5/16 (31%) ASD patients had negative/sub-optimal response while all the HCs were positive (p=0.0184).

IFN-γ level was significantly lower in ASD patients compared to HCs (0.4845±0.2655 vs 1.516±0.2218 IU/mL, p=0.0032). Eight/16 (50%) ASD patients vs 16/17 (94%) HCs developed a cellular response (p=0.0066).

Three/16 (19%) ASD patients did not develop both, humoral and cellular response.

Eight/16 (50%) ASD underwent immunosuppressants (ISs) in the 6 months before the booster. All the 5 ASD patients with a negative humoral response were treated with ISs (p=0.025) while 5/8 (62%) subjects with a negative cellular response underwent ISs.

CONCLUSIONS

ASD patients showed impaired immunogenicity compared to HCs after the COVID-19 vaccine booster, regarding both, humoral and cellular response. The lower response, probably due to ISs, makes these subjects at high risk for COVID-19 complications, in particular, those that did not develop humoral and cellular immunity.

Therefore, tight monitoring/preventive treatments are needed in ASD patients non-responders to vaccine.

P065

NOVEL MECHANISMS THROUGH WHICH METFORMIN INDUCES INHIBITORY EFFECTS IN PRIMARY BREAST CANCER CELLS

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BACKGROUND-AIM

Metabolic traits like type 2 diabetes (T2D), hyperinsulinemia, dysglycemia and obesity, are important factors contributing to breast cancer (BC) progression and metastasis. For instance, high levels of circulating insulin and the cognate receptor, namely insulin receptor (IR), are associated with increased incidence, resistance to therapies and poor outcome in BC patients. Of note, pre-clinical and clinical studies have demonstrated that the first line treatment for T2D, named metformin (1,1-dimethylbiguanide hydrochloride), may elicit anti-cancer effects in diverse tumor types, including BC.

METHODS

To provide novel mechanistic insights on the inhibitory effects of metformin in BC, we performed bioinformatics analysis, TaqMan Gene Expression Assay and gene silencing experiments, immunoblots, immunofluorescence, flow cytometry, proliferation, clonogenic and spheroid formation assays, migration and matrigel drops evasion assays, using as model systems a naturally immortalized BC cell line (named BCAHC-1) and cancer-associated fibroblasts (CAFs) derived from BC patients.

RESULTS

Metformin was able to counteract the activation of transduction pathways, gene expression changes and the proliferation of BCAHC-1 cells triggered by insulin. Of note, metformin prevented the insulin-dependent expression of the metastatic gene CXC chemokine receptor 4 (CXCR4), which has been associated with poor disease-free survival in BC patients characterized by high IR levels. Next, metformin suppressed the CXCR4-mediated invasive phenotype of BCAHC-1 cells prompted by the insulin-stimulated cooperation between cancer and CAFs.

CONCLUSIONS

Our results provide novel insights regarding the inhibitory action of metformin on the proliferative and invasive responses induced by the insulin/IR signaling in both BC cells and important components of the tumor microenvironment like CAFs. These findings may further support the ability of metformin to counteract the stimulatory effects of the insulin/IR axis, particularly in BC patients affected by metabolic disorders.

P066

IL-8 IS INVOLVED IN THE STIMULATORY ACTION EXERTED BY AGES/RAGE AXIS IN BREAST CANCER MICROENVIRONMENT

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BACKGROUND-AIM

Metastatic evolution, which involves multiple interactions between cancer cells and the surrounding microenvironment, is a main cause of breast cancer-related mortality. In this scenario, Advanced glycation end products (AGEs) and the cognate receptor RAGE contribute to the dysfunctional liaisons between breast cancer cells and the inflammatory tumor microenvironment, toward the acquisition of certain malignant features. However, the molecular mechanism as well as the biological effects triggered by the activation of AGEs/RAGE axis in main components of the tumor stroma, named cancer-associated fibroblasts (CAFs), remain to be fully understood.

METHODS

In order to evaluate the inflammatory landscape regulated by the AGEs/RAGE signaling in primary CAFs derived from breast tumors, we first performed PCR human Chemokine Array. Thereafter, the molecular mechanisms implicated in the AGEs-regulated genes were assessed performing qPCR, immunoblots and gene silencing experiments, promoter studies and ChIP assays. To further characterize the functional interplay between CAFs and breast cancer cells prompted by the AGEs/RAGE transduction pathway, we also performed F-actin staining, migration and invasion assays.

RESULTS

Upon AGEs/RAGE activation, interleukin-8 (IL-8) was found as the most up-regulated pro-inflammatory chemokine in CAFs. In particular, the AGEs/RAGE axis triggered certain signaling cascades leading to c-Fos-dependent regulation of IL-8 in CAFs. Next, using conditioned medium from AGEs-stimulated CAFs, the paracrine activation of IL-8/CXCR1-2 transduction pathway was shown to promote migratory and invasive effects in MDA-MB-231 breast cancer cells.

CONCLUSIONS

Overall, our data show that the AGEs/RAGE circuitry is involved in the functional crosstalk that occurs between breast tumor cells and the surrounding stroma toward the acquisition of aggressive breast cancer features.

P067

A NANOPARTICLE-BASED STRATEGY TO OVERCOME MIR-214-DRIVEN DRUG RESISTANCE IN HYPERPIGMENTED MELANOMA CELLS

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BACKGROUND-AIM

Notably, melanin affects the clinical course of melanoma, being hyperpigmentation related to resistance to radio-, chemo-, immuno-, and targeted therapy. Indeed, melanoma cells undergoing drug resistance show hyperpigmentation. Several efforts have been made to discover the melano-miRs involved in melanoma progression, but, to date, they are still to be defined. miR-214 contributes to melanoma initiation and works as a key hub by coordinating fundamental signaling networks critical for stemness, metastatic dissemination, and therapy resistance. This study aims to verify whether miR-214 promotes hyperpigmented/drug-resistant melanoma phenotypes, and to disclose a new nanoparticle-based strategy to abrogate miR-214 expression and possibly counteract such aggressive phenotypes.

METHODS

Melanoma cells transfected with pCMV-miR-214 plasmid were sorted for GFP, maintained under geneticin pressure and validated for increased miR-214 expression in qPCR. Cellular melanin content was evaluated in spectrophotometry, while ROS levels were quantified by the CellROX flow cytometry kit. TargetScanHuman software suggested putative miR-214 targets involved in melanoma hyperpigmentation which were validated in western blot. Melanoma cell response to therapies was determined by MTT and Annexin V/PI assays.

RESULTS

Murine B16 and human 501Mel melanoma cells over-expressing miR-214 (hereafter called miR-214+ cells) showed an augmented pigmentation associated with chemo- and targeted therapy resistance. miR-214+ cells also show an increased ROS production, possibly related to both increaded pigmentation and drug resistance. Being involved in the anti-oxidant response and also the phenylalanine/tyrosine catabolism (i.e., the main melanin precursors), Glutathione S-transferase Zeta 1 (GSTZ1) was selected among miR-214 putative targets and found down-expressed in miR-214+ cells. Phytochemical-embedded gold nanoparticles were able to significantly reduce miR-214 levels, together with melanin content and ROS, suggesting a potential activity in re-sensitizing miR-214+ cells to therapies.

CONCLUSIONS

This study reveals a positive feedback loop between miR-214 and ROS promoting melanoma hyperpigmentation and therapy resistance and proposes a nanoparticle-based strategy to counteract pigmentation and subsequent onset of resistant phenotypes.

P068

GOLD NANORODS HYPERTHERMIA ENHANCES RADIOSENSITIVITY IN NEAR-INFRARED PHOTOTHERMAL AND X-RAY RADIATION THERAPY

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BACKGROUND-AIM

So called cell-based therapies, treatments in which stem or progenitor cells are induced to home within damaged or cancer tissues, and nanomedicine, which relies on the use of nanoparticles (NPs), are becoming outstanding research areas in personalized tumor therapy. Despite continuous technical advances, the radiation-induced toxic effects in adjacent healthy tissues still represent the dose-limiting factor. The aim of our study is to develop an efficient therapeutic strategy to control tumor growth and progression based on the combination of nanomedicine and cell therapy with radiation therapy.

METHODS

Endothelial colony forming cells (ECFCs), a subtype of Endothelial Progenitor Cells, with inherent tumor tropism capability, were chosen to carry AuNPs to tumor cells and were used in co-culture experiments with unloaded melanoma (M6-A375) or breast cells (MCF7). The long-term cytostatic/cytoxic effects of combined radiotherapy and nano-mediated hyperthermia were evaluated using clonogenic assays while the short-term effects were determined evaluating DNA damage by comet assay and cell cycle arrest and autophagy western blot analysis.

RESULTS

We have shown how the cooperative effect between irradiation and hyphertermia is much more effective in MCF7-ECFcs co-colture than M6-ECFC cells. We observed increased levels of γH2AX, also confirmed by comet assay after the combo treatment. Moreover, the combined treatment induces a significative decrease of lc3, a well known marker of the autophagy process which desensitizes cancer cells to radio therapy.

CONCLUSIONS

AuNPs are confirmed to be as excellent radiosentizers and thus allows to shorten the duration of the tratment and to reduce the radiation doses. The combo treatment of MCF7-AuNP enriched ECFCs inibits autophagy.

P069

REACTIVATION OF A PLACENTAL EPIGENETIC PROGRAM DRIVES INSL4 EXPRESSION IN LUNG CANCER

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BACKGROUND-AIM

NSCLC 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 NSCLC cell lines (e.g. A549) and in nearly 8% of NSCLC patients in which correlates with worst OS. INSL4 is physiologically expressed only in placental trophoblast. Here, we investigate the oncogenic mechanisms of INSL4 in NSCLC.

METHODS

Bioinformatic analyzes were performed using Zenbu 3.0 database, the CCLE, cBioPortal, ChIP ATLAS and the Nascent Transcription Repository database.

RESULTS

At first, a bioinformatic analysis of the INSL4 gene was performed. This analysis showed, upstream of the gene, the presence of a HERV with probable activity on the expression of INSL4. HERVs in the genome are epigenetically silenced by DNA methylation, so we proceeded by analyzing the methylome of 92 NSCLC cell lines and 501 patient samples, this analysis showed that INSL4 overexpression were highly correlated with HERV methylation status upstream of INSL4, suggesting that INSL4 overexpression would therefore be strongly influenced by epigenetic factors. Through the GRO-Seq Analysis approach in A549 it is possible to find reads signals consistent with the INSL4 promoter, but also abnormal reads in the HERV LTR, indicating a functional reactivation of the HERV. Then, through the ChIP-atlas database, we have deeply characterized the epigenetic profile of INSL4 in A549. With Bisulfite-Seq, we found a precise profile of HERV LTR hypomethylation and chromatin accessibility through ATAC-Seq, while at the histone level we found a signature of euchromatin (H3K27Ac) and of active Enhancer (H3K4me2/3, H3K9Ac) at HERV LTR. Comparison of A549 with trophoblast cell lines allowed us to find a precise overlap of this epigenetic profile suggesting the same activation mechanism, physiological in one case and pathological in the other.

CONCLUSIONS

These results underline the crucial role of epigenetic mechanisms on the control of gene expression in cancer and begin to shed light on the reactivation of HERVs in oncogenesis. This new mechanism known as onco-exaptation sees HERVs providing functional Enhancers to oncogenes and candidate them as master regulators of gene expression in cancer.

P070

PROTEOSTASIS AND CELL CYCLE: HOPS/TMUB1 MODIFIER IN CONTROLLING PHASE TRANSITION

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BACKGROUND-AIM

The cell cycle refers to the sequence of events that leads to the proper division of eukaryotic cells. Each event is finely controlled at checkpoints, which are able to slow down the cell cycle to attempt a reset and in case they fail lead the cell to death or to exit the cycle. When a cell successfully eludes these surveillance mechanisms, it acquires the ability to replicate uncontrollably and grow disproportionately, leading to cancer onset. In this scenario, a key role is played by ubiquitin-like modifier, known as HOPS/TMUB1 (Hepatocyte Odd Number Protein Shuttle/Transmembrane and Ubiquitin Like Domain Containing Protein 1).

METHODS

Hops$^{+/+}$ and Hops$^{-/-}$ mouse embryonic fibroblasts (MEFs) were prepared from C57BL/6 mice and Hops$^{-/-}$C57BL/6 mice. Hops$^{-/-}$HeLa cells were obtained using the CRISPR/Cas9 method. Identification of HOPS targets was performed and confirmed by LC–MS, co-immunoprecipitation and immunofluorescence assays. Protein half-life evaluation was performed by treating with cycloheximide.

RESULTS

By comparing mitosis in Hops$^{+/+}$ MEFs and Hops$^{-/-}$ MEFs, it became evident that in the lack of HOPS, the percentage of atypical mitosis increases significantly. HOPS is known to be an essential constituent of centrosome assembly. Accordingly, the localization of HOPS during the cell cycle in HeLa cells is phase specific, suggesting specific role during the cell division. HOPS is a modifier known to influence the half-life of its substrates. Proteomic analysis and immunoprecipitation assays on HeLa cells in mitosis made it possible to identify new HOPS targets (such as RhoA, Anillin, RacGAP1, Plk-1). By comparing Hops$^{+/+}$HeLa and Hops$^{-/-}$HeLa came to light that the half-life of these target is shortened when HOPS lacks, suggesting a direct role to promote targets stabilization. Lastly a time lapse assay confirmed a delay in mitosis in Hops$^{-/-}$HeLa cells, demonstrating an active role played by HOPS in the regulation of its targets, which in turn are crucial for proper cell cycle progression.

CONCLUSIONS

HOPS can be considered a key player that preserves and promotes proper cell division. Its involvement in critical control phases make it a potential candidate in the complex process of tumour escape, opening up new horizons in the cancer response.

P071

DIETARY INTERVENTION AFFECTS SPECIFIC INFLAMMATORY AND METABOLIC BIOMARKERS IN PATIENTS WITH CANCER

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BACKGROUND-AIM

Neoplastic diseases represent the second leading cause of death worldwide. Their patterns' plasticity involves multiple environmental factors. Malnutrition is, in fact, a common feature in cancer patients, which hugely impacts on quality of life, as well as maintaining a state of systemic chronic inflammation. This study aimed to assess how an "anti-inflammatory" dietary intervention may affect specific cytokine levels and metabolic biomarkers in a cohort of neoplastic patients.

METHODS

140 patients (86 females and 54 males) were recruited at Azienda Sanitaria Locale Napoli 3 Sud. For each patient, anagraphic, anamnestic and biochemical data were collected, as well as pathological and therapeutic information about their neoplastic condition. All patients were fed with an "anti-inflammatory" nutrition plan. Serum samples were collected at the beginning of the study and six months later; at both times, samples were screened for the concentration of 28 cytokines, chemokines, and growth factors and 10 metabolism-related factors, both based on a multiplex approach.

RESULTS

Within the 140 subjects, the most frequent neoplastic conditions were: Breast Cancer (BC, n: 53), Colon Cancer (CC, n: 44) and Prostate Cancer (PC, n: 13). After dietary intervention, BC and PC patients showed significant mean reductions for general bioimpedance parameters, such as Body Weight (BC: -4.2 Kg; PC: -6.3 Kg), BMI (BC: -1.45 Kg/m^2; PC: -2.05 Kg/m^2), Fat Mass (BC: -3.12 Kg; PC: -4.8 Kg) and Visceral Adipose Tissue (BC: -1.04 Kg; PC: -2.14 Kg). Moreover, BC patients showed significant reduction of IP-10 (-1006 pg/ml) and Visfatin (-1704 pg/ml), while PC patients had significant reduced levels of MCP-1 (-45.6 pg/ml) and increased levels of Adiponectin (+1.49 mg/l). CC patients did not show any significant difference for general bioimpedance parameters; however, they displayed a robust anti-inflammatory response, with significant mean reductions of cytokines like IL-1b (-6.19 pg/ml), IL-6 (-5.14 pg/ml), IL-8 (-797 pg/ml), MIP-1α (-135 pg/ml), MIP-1β (-408 pg/ml), and TNF-α (-131 pg/ml).

CONCLUSIONS

In neoplastic patients, "anti-inflammatory" dietary intervention might target both cytokines and metabolic biomarkers, with cancer-specific patterns.

P072

DEFINING CELLULAR AND SOLUBLE COMPONENTS INVOLVED IN BONE MARROW CELL-BASED THERAPY IN PATIENTS WITH CRITICAL LIMB ISCHEMIA

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BACKGROUND-AIM

Critical limb ischemia (CLI) is a debilitating cardiovascular disease characterized by chronic ischemic rest pain, ulcers, and tissue loss. More than 30% of patients do not qualify as candidates for surgical or endovascular treatments, and are designated as "no-option" CLI patients. 40% of these patients undergoes major limb amputation, while 60% dies within 6-12 months from the diagnosis. Bone marrow (BM) cell-based regenerative therapies have been proposed as novel treatment strategies; however, their mechanisms are still unclear.
The aim of this pilot study is to define the cellular and soluble components of blood and BM concentrates (BMC) obtained at the time of BMC therapy in patients with CLI, and to evaluate a possible association between these factors and patient clinical outcome upon 30 days.

METHODS

10 male CLI patients selected for BMC therapy were enrolled. Antropometric and clinical (transcutaneous oxygen tension-$TcpO_2$, and treadmill test-TT) data were collected at the day of the therapy (T0) and after 30 days (T1). ΔTT and $\Delta TcpO_2$ were calculated as T1 -T0. BMC samples were obtained employing Sepax-2 technology. Peripheral blood (PB) samples were also collected. Endothelial progenitor cells –EPC ($CD45^{dim}$, $CD34^+$, $CD133^+$ cells) and mesenchymal stem cells-MSC ($CD45^-$, $CD90^+$, $CD73^+$ cells) were analyzed by flow cytometry. A panel of cytokines, chemokines and growth factors was evaluated by multiplex ELISA.

RESULTS

BMC contained 3-fold higher number of cells compared to BM aspirates. In BMC, EPC represented the 50% of the $CD45^{dim}$ cells, a percentage significantly higher compared to that observed in PB. MSC represented the 0.3% of $CD45^-$ cells both in BMC and in PB. BMC had a significantly higher concentration of IL-6, CCL5 and PDGF compared to PB. Moreover, in PB the concentration of IL-5, IL-6 and eotaxin negatively correlated with ΔTT, while the concentration of PDGF positively correlated with $\Delta TcpO_2$. Finally, in BMC, ΔTT established a significant strong positive correlation with PDGF and FGF concentrations. Interestingly, MSC percentage, PDGF and IL-2 concentrations positively correlated with $\Delta TcpO_2$.

CONCLUSIONS

BMC therapies rely on specific cellular components, such as the MSC, and soluble factors, such as the PDGF, whose interplay drive the patient clinical outcome.

P073

IGG GLYCOSYLATION IN ENZYME-LINKED IMMUNOSORBENT ASSAY SET UP

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BACKGROUND-AIM

Sensitivity of ELISA assays can be increased by the orientation of antibodies (IgGs) on capturing surfaces. IgGs orientation can be achieved by making the thiol groups of hinge regions available by suitable reduction conditions thus obtaining half fragment IgGs (rIgGs) in which the antigen binding site is preserved. rIgGs will interact with surfaces coated with maleimide or gold. Differences in the reduction susceptibility were observed among mouse monoclonal IgGs of the same subclass. Indeed, it has been shown that the tertiary structure of Fc fragments changes accordingly to the glycosylation pattern of the Fc constant immunoglobulin domains CH2.
Aim of this work is to investigate if the glycosylation pattern affects the susceptibility of IgG hinge regions to chemical reduction.

METHODS

Purified mouse anti-fPSA IgGs were deglycosylated using the enzyme EndoS, an endoglycosidase specific for cleaving the N-linked glycans from IgGs heavy chains. Glycosylated and deglycosylated IgGs were reduced by 90 min incubation at 37°C in presence of 53mM 2-MEA solubilized in PBS containing 10mM EDTA. Deglycosylation and reduction were verified by 8% SDS-PAGE in presence or not of β-mercaptoethanol. Protein bands were stained by blue Coomassie. Deglycosylated rIgGs were oriented on maleimide microplates to perform a sandwich ELISA for the detection of PSA.

RESULTS

Electrophoretic band patterns from SDS-PAGE containing β-mercaptoethanol showed that glycosylated anti-fPSA was resistant to reduction while deglycosylated anti-fPSA was completely reduced to heavy and light chains. Both glycosylated and deglycosylated anti-fPSA were reduced using 2-MEA: non-reducing SDS-PAGE revealed that rIgGs could be obtained only from deglycosylated anti-fPSA. Oriented ELISA sandwich based on deglycosylated and reduced anti-fPSA allowed a two-fold LoD enhancement in comparison with the corresponding non oriented assay based on whole IgGs (0.004 ng/ml vs. 0.011 ng/ml).

CONCLUSIONS

Results confirmed that glycosylation affect the susceptibility of anti-fPSA to chemical reduction. So, deglycosylation of IgGs could allow the standardization of the chemical reduction improving the reproducibility and applicability of this method for the orientation of IgGs on capturing surfaces.

P074

DNA METHYLATION DYNAMICS AT GENES SPECIFIC LEVEL DURING BRAIN DEVELOPMENT AND IN SCHIZOPHRENIA

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BACKGROUND-AIM

Defined epigenetic modifications occurring during brain development may play a fundamental role on brain function. An alteration in the establishment of correct DNA methylation at specific genes has been associated with neuropsychiatric disorders. During perinatal period DNA methylation may finely control genes regulating brain levels of critical neuromodulators such as D-Serine and D-Aspartate. Since levels of these D-amino acids have been found altered in some mental disorders the lack of an epigenetic control may contribute to the genesis and/or progression of these diseases

METHODS

We performed a comprehensive DNA methylation analysis along with mRNA expression at DAO and DDO genes, involved in the degradation of D-Serine and D-Aspartate, respectively, in mice during brain development and in post-mortem tissues of patients with schizophrenia. We evaluated DNA methylation using amplicons bisulfite sequencing and performed an in-depth single molecule methylation approach in order to assess the cell to cell methylation heterogeneity.

RESULTS

We found strong spatiotemporal changes in DNA methylation during development, especially in cerebellar astrocytes and at specific CpG sites. These CpGs showed high degree of 5-hmC at P1 and dramatically underwent demethylation during development. This demethylation strongly activated gene expression, indirectly promoting the physiological degradation of D-aminoacids. The present study demonstrates for the first time that D-Serine and D-Aspartate levels during brain development are indirectly regulated by DNA methylation that govern the expression of DAO and DDO genes. Furthermore, single-molecule methylation approach promises to identify different cell-type composition and function in different brain areas and developmental stages.

CONCLUSIONS

In conclusion, we hypothesize that eventual dysfunction of postnatal changes of DNA methylation/hydroxymethylation dynamics at few CpG sites, indirectly establishing the proper D-Ser and D-Asp brain levels, may have profound clinical impact in the etio-pathogenesis and treatment of neurodevelopmental disorders.

P075

DEFINING THE ROLE OF THE LYSINE METHYLTRANSFERASE SETD8 AS A NOVEL EPIGENETIC TARGET FOR THE THERAPY OF GLIOBLASTOMAS

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BACKGROUND-AIM

Glioblastoma multiforme is a largely incurable brain tumor.Regardless of recent progresses in the characterization and classification of glioblastomas, very few progresses have been made in the individuation of new effective drugs, despite intense searching. To this end, we analyzed mRNA expression in glioblastomas, utilizing both publicly accessible databases and our own RNA-seq data, focusing our attention at differentially expressed RNA coding for proteins involved in epigenetic processes, using normal brain tissue as control. We found mRNA coding for the lysine methyltransferase SETD8 to be highly overexpressed in glioblastomas. SETD8 was first identified as the sole mammalian enzyme able to catalyze the mono-methylation of histone H4 on lysine 20, a post-translational, epigenetic modification key for DNA damage repair. To date, there are no published data about SETD8 in glioblastomas. Thus, the aim of our study was investigate protein levels and function of SETD8 in glioblastoma.

METHODS

To verify SETD8 protein levels in GBMs tissue, we perform IHC on microarray. To study the role of SETD8 in GBMs we utilized GBM cell lines and performed inihibiition utilizing a specific SETD8 inhibitor drug. We performed FACS analyses, IFs and WBs to validate cell cycle arrest and DNA damage. To demonstrate that SETD8 inhibitor and Wee1 inhibitor cause cell death we performed MTT and caspase activity tests. Death through mitotic catastrophe was elucidate performing time lapse experiments.

RESULTS

We observed that SETD8 is overexpressed in 47% of the gliomas we analyzed. Going further, our evidence proves a functional role for SETD8 in glioblastoma. A specific SETD8 inhibitor, UNC0379, blocks glioblastoma cell proliferation, by inducing DNA damage and, in turn, safeguarding activation of the cell cycle checkpoints.The abrogation of the G2/M checkpoint by a Wee1 kinase inhibitor, AZD1775, induces the glioblastoma cell lines, DNA-damaged by UNC0379, to progress to mitosis where they die by mitotic catastrophe. Thus, our preliminary data indicate the association UNC0379+AZD1775 as a successful strategy for glioblastoma treatment, especially for those overexpressing SETD8

CONCLUSIONS

In this scenario, SETD8 is a biomarker, easily investigated by immunohistochemistry, for predicting response to a novel association of drugs: a targeted SETD8 inhibitor + a targeted Wee1 inhibitor.

P076

ROLE OF EXTRACELLULAR VESICLES (EVS) IN THE MODULATION OF PRO-TUMORAL SENESCENT FIBROBLAST SECRETORY PHENOTYPE (SASP) BY POLYPHENOLS.

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BACKGROUND-AIM

Senescent cells are reported to show increased secretion of extracellular vesicles (EVs), characterized by altered protein composition and pro-proliferative function on some cancer cell lines. Previous data have reported the protective effects of chronic treatment with resveratrol, starting from modulation of SASP-factors secretion by senescent neonatal human dermal fibroblasts (NHDFs). On these bases, we studied the role of the chronic resveratrol treatment in the modulation of EVs from senescent NHDFs and the role of EVs in tumor cells proliferation.

METHODS

Senescence NHDFs were treated for 5 weeks with resveratrol 5μM(R5). Untreated and R5 treated conditioned medium (CM) from senescent NHDFs were collected for EVs isolation, through two steps of ultracentrifugation. The effect of R5 treatment on the EVs modulation were evaluated analyzing the size, count rate and expression of specific EVs markers. Cancer cell lines (A375-M6 and MCF7) were incubated for 48 hours with CMs untreated and R5 treated, both complete and depleted of EVs.

RESULTS

Our results evidenced that R5 treated CMs from senescent NHDFs showed an increase of exosomal population, compared to untreated CMs; we also demonstrated a reduction of proliferation activity in A375-M6 and MCF7, incubated with R5 treated CMs complete of EVs, compared to untreated CMs from senescent NHDFs. These data suggest that R5 treatment modulate composition of senescent EVs and exert a protective effect on pro-proliferative function of some cancer cells

CONCLUSIONS

Next experiments will focus on molecular mechanisms and factors of EVs from senescent cells that modulate tumor cell proliferation. Furthermore, we will evaluate the presence of resveratrol in EVs, to implicate EVs as nanovectors for delivery of agents to new possible therapeutic approaches for cancer and senescence diseases.

P077

EVIDENCE THAT ADIPONECTIN UP-REGULATES E-CADHERIN EXPRESSION IN ERα+ BREAST CANCER CELLS, PROMOTING TUMOR GROWTH, PROGRESSION, AND DISTANT METASTASIS

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BACKGROUND-AIM

Adiponectin (Adipo) is the major adipocytes-secreted protein involved in obesity-related breast cancer (BC). We proved that Adipo promotes proliferation in ERα+ BC cells, through ERα transactivation. Moreover, Adipo induces LKB1 recruitment as ERα-coactivator, impairing LKB1/AMPK signaling activation. Here, we showed that Adipo-mediated ERα transactivation enhances E-cadherin (E-cad) expression. Thus, we investigated the molecular mechanism through which ERα/LKB1 complex may modulate the expression of E-cad, influencing tumor growth, progression, and distant metastasis

METHODS

qRT-PCR and immunoblot for E-cad expression. Functional studies on E-cad promoter were done by transient transfection and ChIP assays. Cell-cell interaction was studied in 3D model. Immunofluorescence assay (IF) for proteins distribution. ERα or LKB1 action was also tested in the presence of specific siRNAs. In vivo studies were performed using orthotopic xenografts model and metastasis assay

RESULTS

Our results demonstrated that Adipo signaling enhances E-cad content in MCF-7 cells, through the activation of its gene promoter by ERα/LKB1complex. This was confirmed by transient transfection with E-cad promoter and ChIP assays. The enhanced E-cad expression impacted on an increased cell-cell adhesion and proliferation tested in Adipo-treated 2D and 3D MCF-7 cultures. It is well known that E-cad connects cell polarity and cell growth driving the proper localization of many proteins, like LKB1 and Cdc42 in polarized region. IF showed that in Adipo-treated MCF-7 cells LKB1 and Cdc42 colocalized in the nucleus, impairing their cytosolic cooperation in maintaining cell polarity, as confirmed by Golgi apparatus orientation. None of these effects were noticeable in BT-20 cells. The orthotopic implantation of MCF-7 cells revealed an enhanced E-cad-mediated BC growth induced by Adipo. Moreover, tail vein injection showed a higher metastatic burden in the lungs of mice receiving Adipo-treated MCF-7 cells compared to BT-20 cells

CONCLUSIONS

Based on our data, it emerges that in ERα+ BC upon Adipo exposure E-cad becomes a tumor growth stimulating factor, altering cell polarity, and promoting distant metastasis. All this addresses that the role of E-cad generally assumed as tumor suppressor of BC needs to be carefully reassessed

P078

DISSECTING THE PRO-TUMORIGENIC ROLE OF INTERLEUKIN (IL)-11 BY BOTH TRANSCRIPTOMIC ANALYSIS OF PRIMARY BREAST CANCER CELLS AND LARGE-SCALE IN SILICO INVESTIGATIONS

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BACKGROUND-AIM

The advent of high-throughput methodologies along with the availability of multi-omics datasets are crucial to define at molecular level heterogeneous diseases, including breast cancer (BC). We aimed to dissect transcriptional changes mediated by estrogen and insulin in a primary BC cell line, namely BCAHC-1, which is characterized by a peculiar expression of the 46 kDa isoform of estrogen receptor (ER)α and the insulin receptor (IR).

METHODS

The Bcl2Fastq 2.20 version of the Illumina pipeline was used to process raw data obtained from RNA sequencing (RNA-seq) analysis of BCAHC-1 cells. Bioinformatics analysis on the most induced gene by both estrogen and insulin named interleukin (IL)-11 were accomplished using mRNA expression data and clinical information of the TCGA dataset in R Studio. Samples were filtered by the "sample type" to separate BC tissues from matched non-tumor tissues and by the expression of ER. The survival analyses were performed on IL-11 expression levels and the disease-specific survival (DSS), disease-free interval (DFI) and progression-free interval (PFI) information, employing the survminer and survivALL packages. The cor.test() function and the ReactomePA package served for correlation and pathway enrichment analysis, respectively.

RESULTS

Analyzing through RNA-seq the differentially expressed genes in BCAHC-1 cells exposed to E2 and insulin, we assessed that the pro-inflammatory cytokine IL-11 is the most up-regulated gene upon exposure to these hormones. Through bioinformatics analysis on the TCGA cohort we ascertained that IL-11 is both significantly over-expressed in ER-positive BCs respect to adjacent normal tissues and associated with worse DSS, DFI and PFI in ER-positive BCs. Interestingly, using the top IL-11 positively correlated genes we determined by pathway enrichment analyses the involvement of this cytokine in metastasis-related paths.

CONCLUSIONS

Our data suggest that IL-11 may play a role in ER-positive BC progression. Additional in vitro and in vivo investigations are warranted in order to assess the usefulness of therapeutic strategies targeting IL-11 in BC.

P079

THE ENDOTHELIAL TRANSCRIPTION FACTOR ERG MEDIATES A DIFFERENTIAL ROLE IN THE ASCENDING AORTA, DILATATED OR ANEURYSMATIC, WITH BICUSPID OR TRICUSPID AORTA VALVE

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BACKGROUND-AIM

Recent genome-wide association studies and other current works have suggested the key role of ERG gene [erythroblast transformation-specific] related gene) in maintaining aortic wall function. ERG, precisely, promotes endothelial homeostasis, including regulation of proliferation, survival, and barrier function, and the consequent vascular stability, by controlling a wide range of targets and pathways, including TGF-β-SMAD 3 involved in fibrosis's mechanism. ERG modulates canonical TGF-β-SMAD signaling to reprime vascular fibrosis, by inhibiting SMAD3 activity and activating SMAD1. This results in a control of EndMT and the rate of SMAD 3 mediated fibrogenesis. Based on this evidence, we aimed to assess if ERG is involved in EndoMT mechanism, fibrosis, and calcification of aneurysmatic ascending aortas (AAA) with bicuspid or tricuspid valve (BAV, TAV) on a broad basis to first get a complete identification on the potential differences in expression and mediated roles among the diverse groups (with AAA or not) of BAV and TAV cases included in the study.

METHODS

To achieve such main aim, our study was complemented by histological and immunohistochemical investigations on tissue aorta samples from four groups (BAV, BAV with AAA, TAV and TAV with AAA), as well as by an extensive real time PCR-based gene expression analyses

RESULTS

The data obtained demonstrated that BAV cases with AAA had a higher percentage of ERG+ endothelial cells (EC) in their tissue aorta samples, accompanied by a significant gene ERG expression than non-aneurysmatic BAV and TAV groups. In the contrary, the levels of SMAD3 were significantly higher in EC and VSMC from TAV cases with AAA. A higher number of αSMA+/S100A4 EC and VSMC in TAV aorta samples with AAA, was also detected, as well as the highest rate of fibrosis evidenced by Masson's trichrome staining. While BAV with AAA also showed a significant up-expression of miR126, involved in inhibiting EnMT transition by targeting SMAD3/2 genes, that significantly correlated with the ERG levels in gene and tissues expression. They also had a higher rate of calcification in their aorta tissues, evidenced by Alizarina standing.

CONCLUSIONS

Thus, ERG appears as novel EC-specific regulator of specific targets and pathways of aorta, as well as of the progression of its diseases, i.e. AAA, and consequently an optimal biomarker and target.

P080

EFFICACY OF PROBIOTIC STREPTOCOCCUS THERMOPHILUS IN COUNTERACTING TGF-β1-INDUCED FIBROTIC RESPONSE IN HUMAN DERMAL FIBROBLASTS VIA INHIBITING SMAD SIGNALING.

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BACKGROUND-AIM

Abnormal and deregulated skin wound healing associated with prolonged inflammation may result in dermal fibrosis, such in hypertrophic scarring and keloids, characterized by dysregulated deposition of extracellular matrix in the dermis, leading to skin function and architecture impairment. The rise of hypertrophic scars can dramatically diminish the life quality of patients. Unfortunately, the current therapeutic strategies are unsatisfactory. In this study, we aimed to evaluate whether the lysate from S. thermophilus could antagonize the fibrogenic effects of TGF-ß1 in normal human dermal fibroblasts (NHDF).

METHODS

NHDF were exposed to TGF-ß1 to establish a fibrotic phenotype. Proliferation rate and cell number were measured using the IncuCyte® Live Cell Imager system and the trypan blue dye exclusion test. Phenoconversion markers (α-SMA and fibronectin) and collagen I levels were assessed by Western blot and immunofluorescence. The mRNA levels of TGF-ß1 were evaluated by RT-PCR. The Smad2/3 phosphorylation level as well as ß-catenin and PPARγ expression, were assessed by Western blot. The cell contractility function of NHDF and cell migration were studied using collagen gel retraction assay and, scratch wound healing assay, respectively. The effects of S. thermophilus lysate, alone or combined with TGF-ß1, were evaluated on all the above-listed parameters and markers associated with TGF-ß1-induced fibrotic phenotype.

RESULTS

The addition of S. thermophilus significantly reduced the cellular and molecular features associated with the fibrotic phenotype in TGF-ß1-activated NHDF. The results showed the evident ability of the probiotic to antagonize the fibrogenic effects of TGF-ß1 by inhibiting Smad-signaling. The Wnt/ß-catenin pathway was also shown to be suppressed by S. thermophilus, and this effect correlated with the PPARγ level increase.

CONCLUSIONS

This is the first report showing that S. thermophilus lysate had a remarkable anti-fibrotic effect in TGF-ß1-activated NHDF through inhibiting Smad signaling and the Wnt/ß-catenin pathway. The probiotic lysate modulated the ß-catenin levels negatively and PPARγ expression positively. Our findings support that S. thermophilus may help prevent or treat hypertrophic scarring and keloids.

P081

AMOEBOID ANGIOGENESIS: A NOVEL ESCAPE MECHANISM FROM ANGIOGENESIS INHIBITOR THERAPY

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BACKGROUND-AIM

In the past decades antitumoral drugs inhibiting matrix metalloproteinase (MMPs) were developed to stop tumor progression. However, results from human clinical studies were disappointing. Individual cells are able to invade using two different invasive methods known as mesenchymal or amoeboid. Mesenchymal motility requires an elongated cell morphology and the ability to degrade ECM by MMPs. The amoeboid movement does not utilize MMPs and adapts cell shape to glide through preexisting gaps. Recently, we first demonstrated that endothelial cells (EC) can switch between invasion modes in order to cope with challenging environments, performing the "amoeboid angiogenesis" in the absence of proteases activity.

METHODS

Matrigel invasion assay showed that the most high-powered protease inhibitors able to induce the amoeboid invasion capacity of ECs were TIMP1, 2 and 3. To confirm if the ECM degradation inhibition by MMPs was responsible for the switch of endothelial cells from mesenchymal to amoeboid invasion strategy, and not related to any protease inhibition-independent activity of TIMPs, we used Marimastat, a broad-spectrum MMP inhibitor used in clinical.

RESULTS

Morphological studies, invasion assay, capillary morphogenesis assay, and biochemical evidences confirmed that Marimastat, instead of inhibiting as previously thought, promotes the invasion and tubular formation of ECs inducing amoeboid characteristics. Thus, once the mesechymal-amoeboid shift was triggered by Marimastat, we explored VEGF role in amoeboid angiogenesis inhibiting its activity with Bevacizumab. We observed that the combination of Marimastat plus VEGF doesn’t boost neither cell invasion nor vessel formation capacity. Moreover, cell treatment with Bevacizumab in the presence of Marimastat confirmed that the amoeboid angiogenesis is independent from the stimulus of the main vascular growth factor, VEGF.

CONCLUSIONS

Therefore, the results obtained could justify the failure of synthetic metalloproteinase inhibitors as cancer therapy and explain the tumor resistance to VEGF-targeted therapies as result of the overcome of amoeboid cancer and endothelial cell behavior. Targeting both VEGF and amoeboid movement in both endothelial and cancer cells, should result in more effective tumor growth inhibition.

P082

MESENCHYMAL STROMAL CELLS SUSTAIN MULTIPLE MYELOMA CELLS GROWTH BY TRADING GLUTAMATE FOR GLUTAMINE

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BACKGROUND-AIM

Multiple myeloma (MM) is a glutamine (Gln)-addicted cancer and lacks Gln Synthetase (GS), the only enzyme able to synthesize Gln from glutamate (Glu). Thus, MM cells avidly take up extracellular Gln through the activity of ASCT2 transporter and create a peculiar low-Gln, high-Glu metabolic niche in the bone marrow (BM) plasma of MM patients. Gln depletion impacts on tumor microenvironment, impairing osteoblast (OB) differentiation of mesenchymal stromal cells (MSC) and favouring bone lesions, typical of MM. Moreover, MSC readily adapt to Gln starvation increasing GS expression, suggesting that they may sustain MM cell metabolism. This hypothesis is assessed in this contribution.

METHODS

Primary human BM MSC were cultured in DMEM for no more than five passages in monoculture or co-culture with Human Myeloma Cell Lines (HMCL). MSC were incubated with osteogenic factors for 14 days, and differentiation was evaluated from the expression of OB markers. Stable isotope tracing was performed by mass spectrometry. Gene Expression Profiles (GEP) were performed on primary human BM MSC and OB from bone biopsies of both healthy donors (n=7) and MM patients (n=16).

RESULTS

HMCL secrete high amounts of Gln-derived Glu and express the efflux Glu transporter Xct, whose expression increases during MM progression. Moreover, MSC actively take up Glu through the inward EAAT3 Glu transporter, the expression of which expression is further increased under hypoxic conditions typical of BM. GEP of MSC and OB reveal that the expression of EAAT3 transporter is higher in MSC compared to OB. Consistently, radiolabeled Glu uptake is higher in MSC than in OB. In Gln-free conditions and in the presence of $^{15}N\text{-}NH_4^+$, MSC produce and secrete ^{15}N-Gln, whose secretion is 5-fold increased when Glu is added in the extracellular space. Glu supplementation increase also α-ketoglutarate secretion by MSC. Finally, in co-cultures of HMCL and MSC, the Gln-efflux transporter SNAT5, as well as GS, are induced in MSC, while MM cell growth is stimulated by MSC in Gln-restricted condition.

CONCLUSIONS

We conclude that GS-negative MM cells secrete Glu used by MSC to produce and release Gln, which, in turn, sustains malignant growth. Thus, a pro-tumor Glu/Gln cycle exists in MM BM and represents a potentially druggable metabolic pathway.

P083

ESSENTIAL GENE SCREENING AND FUNCTIONAL CHARACTERIZATION IDENTIFIES NEW THERAPEUTIC TARGETS AGAINST ENDOCRINE THERAPY-RESISTANT BREAST CANCER

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BACKGROUND-AIM

Targeting vulnerabilities of cancer cells to identify key genomic regulators of cell proliferation and survival (fitness genes) represents a promising way to identify new therapeutic targets and to overcome resistance to current therapies. In breast cancer (BC), endocrine therapy (ET) resistance arises in most cases from constitutively active or aberrant Estrogen Receptor alpha (ERα) signaling and identifying fitness genes within the ERα pathway represents a rational for the development of effective new treatments against these deadly tumors.

METHODS

We combined bioinformatics analysis of genome-wide CRISPR-Cas 'drop-out' screenings and interactome profiling, siRNA-mediated gene knock-down, transcriptome profiling and pharmacological inhibition to cellular and functional assays in antiestrogen (AE)-sensitive and -resistant human BC cell models to identify a set of fitness genes in luminal-like, ERα+ BC cells and characterize their involvement in hormonal signaling, cell proliferation and survival.

RESULTS

A set of essential genes involved in key BC functions was discovered, including some enconding bromodomain-containing proteins belonging to a family of epigenetic "readers" that act as chromatin remodeling factors to control gene transcription. Specific inhibitors of these factors block proliferation of AE- (tamoxifen or fulvestrant) sensitive and resistant BC cells via transcriptome changes that result, among others, on inhibition of hormone, cell cycle and epithelial-to-mesenchymal transition pathways.

CONCLUSIONS

Identification of a functional interplays between ERα and key components of its signaling pathway, and their impact on cell proliferation and survival in ET-resistant BC cells reveals new actionable therapeutic targets for treatment of these aggressive tumors.

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P084

A QUANTITATIVE STUDY ON PORPHYROMONAS GINGIVALIS ABUNDANCE IN ORAL CAVITY AND THE AMOUNT OF SERUM ANTIBODIES IN NEUROINFLAMMATORY AND/OR NEURODEGENERATIVE DISEASES

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BACKGROUND-AIM

Porphyromonas gingivalis (PG), a major subgingival plaque bacterium in periodontitis, has recently been associated to neuroinflammatory and neurodegenerative disorders as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis.

The present study aimed to quantify Pg abundance in patients affected by neuroinflammatory and/or neurodegenerative diseases compared with controls without neurological diseases, to determine possible association between Pg abundance and neurodegenerative process.

METHODS

Pg abundance was quantified on DNA extracted from oral cavity samples by quantitative polymerase chain reaction (qPCR). Anti-Pg antibodies were also detected in serum samples by enzyme-linked immunosorbent assays (ELISA). Case series consisted of 35 controls (mean age 53.8 ± 18.9, 49% males) and 35 patients (mean age 68.4 ± 15.6, 51% males) affected by neuroinflammatory and/or neurodegenerative diseases.

RESULTS

The Pg abundance in the oral cavity was higher in patients than in controls ($p= 0.02$), but the anti-Pg antibodies quantity was not significantly different between the two groups ($p=0.83$). The Pg abundance was correlated with the Pg-antibodies amount in control group ($p=0.0001$) and in patients' group ($p = 0.0001$), but with significant different slopes between the two groups ($p = 0.00005$). Due to the significant difference on age between controls and patients ($p=0.001$) the age factor was included as covariate. Results showed that Pg abundance in the oral cavity as well as the anti-Pg antibodies quantity was not related to age factor ($p=0.42$ and $p=0.93$, respectively).

CONCLUSIONS

These results confirm the hypothesis that oral pathogens can induce an host immune response, influencing the progression of the disease. They also suggest an interaction between local inflammation and a state of chronic neuroinflammation and/or neurodegeneration.

P085

EFFECTS OF FUSOBACTERIUM NUCLEATUM ON MIGRATION AND CYTOKINES PRODUCTION OF HUMAN GASTRIC ADENOCARCINOMA AGS CELL LINE

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BACKGROUND-AIM

Gastric cancer is the fifth most frequently diagnosed cancer and is the third leading cause of cancer death worldwide. Although Helicobacter pylori (Hp) infection is the most important risk factor for gastric cancer, it is clearly known that the etiology of the tumor is multifactorial, since only 1-3% of infected patients develop cancer. Therefore, attention is focused on the role of the gastric microbiota in contributing to the progression of tumorigenesis, as H. pylori is lost in its most advanced stages. Indeed, in some studies an alteration of the microbiota in gastric cancer has been shown. Among the various components of the microbiota, Fusobacterium nucleatum (Fn) has been found in in biopsies of patients with gastric cancer.
The aim of this study was to evaluate the effects of Fn infection on a model of human gastric adenocarcinoma AGS cell line in order to verify whether it affects metastatic behavior.

METHODS

Confocal microscope was used to evaluate the Fn localization and the effects on AGS cells morphology. After infection with Fn the effect on migration of AGS cells was determined by wound healing assay. Finally, the impact of the infection on cytokines expression and quantification was evaluated by Luminex assay.

RESULTS

The results obtained showed that Fn co-localized at level of the plasma membrane demonstrating the ability of Fn to adhere to AGS cells. In addition, increases in incubation times were associated with its intra-cellular localization with loss of the classic curved rod shape. Interestingly, Fn determined a greater capacity of cell migration compared to untreated AGS cells. Moreover, our findings demonstrated that Fn modulates IL-4 cytokine expression that could be involved in enhanced cell migration capability.

CONCLUSIONS

Future studies are needed to characterize how Fn affects cell signaling pathways during cancer metastasis that will lead to targeted approaches that could potentially reduce the risk of progression to gastric cancer.

P086

CYTOTOXIC EFFECT OF 5-AMINOLEVULINIC ACID (5-ALA) IN A NOVEL GEL FORMULATION AND PHOTODYNAMIC THERAPY (PDT) ON ORAL AND PANCREATIC CANCER CELL LINES

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BACKGROUND-AIM

Oral and pancreatic carcinomas are among the most aggressive cancers. 5-ALA induces the production of Protoporphyrin IX (PpIX), an endogenous photosensitizer in the heme biosynthetic pathway. Photodynamic Therapy (PDT) is based on the reaction between light activated PpIX and molecular oxygen, which leads to cell death due to an increase in intracellular reactive oxygen species (ROS). Administration of exogenous 5-ALA improves the production of PpIX by cancer cells, increasing the rates of cell death. Aladent (ALAD) is a medical device consisting of a gel containing 5% 5-ALA capable of easily penetrating cell membranes. This study intends to test the effect of ALAD-PDT on oral (CAL-27) and pancreatic (CAPAN-2) cancer cell lines.

METHODS

CAL-27 and CAPAN-2 cell lines were incubated with ALAD in different concentrations: 0.05% (0.23 mM), 0.2% (0.92 mM), 0.4% (1.84 mM), 0.75% (3.45 mM), 1% (4.6 mM), 1.5% (6.9 mM) for 2, 3, 4, 8 hours. Finally, the cells were irradiated by a 630nm ± 10nm FHWM wavelength LED device (TL-01). The cytotoxic effects of ALAD-PDT were measured using the MTS assay. These results were compared with untreated and non-irradiated cells. To evaluate the selective toxicity of ALAD-PDT we carried out the same treatment on normal keratinocyte cells (HaCat). Apoptosis, cell cycle, and ROS production were also evaluated.

RESULTS

The MTS assay showed the highest cell mortality rate for CAL-27 treated with ALAD-PDT at 1.84 mM for 8 hours (79.3%) and for CAPAN-2 treated with ALAD-PDT at 0.23 mM for 4 hours (99.3%). The cytotoxic effect was not observed on non-ALAD-treated and non-irradiated cells. On HaCat cell lines, cell viability is 54.4% when treated with ALAD at 1.84 mM for 8 hours and 60.8% when treated with ALAD at 0.23 mM for 4 hours. The apoptosis assay showed the highest rate of dead cells in CAL-27 after treatment with 1.84 mM ALAD (47.5%) and in Capan-2 with a concentration of 3.45 mM ALAD (32.1%). Cell cycle variation was observed in CAL-27 but not in CAPAN-2. Finally, an elevated ROS production was detected in both cell lines (40% in CAL-27 and 70% in CAPAN-2).

CONCLUSIONS

ALAD-PDT influences the growth of CAL-27 and CAPAN-2 cells in vitro, suggesting in ALAD a new candidate for the clinical treatment of oral and pancreatic cancer.

P087

FOXO3A RESTORES ANTIESTROGEN SENSITIVITY THROUGH THE METABOLIC REPROGRAMMING OF TAMOXIFEN RESISTANT BREAST CANCER CELLS

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BACKGROUND-AIM

Tamoxifen resistant breast cancer cells (BCCs) are characterized by an enhanced metabolic phenotype compared to tamoxifen sensitive breast cancer cells. The PI3K/Akt downstream target FoxO3a has been described as an important modulator of cell metabolism and its deregulation has been involved in the acquisition of resistance to tamoxifen treatment. Therefore, FoxO3a role in the reversion of the metabolic alterations of antiestrogen-resistant cells was investigated.

METHODS

FoxO3a was overexpressed in MCF-7 (parental) BCCs and in the deriving tamoxifen resistant cell line (TamR). Both parental and TamR cells were subjected to proteomic analysis, while seahorse assay was employed to evaluate the extra cellular acidification rate ECAR and oxygen consumption rate OCR. Enzymatic activities of LDH and G6PDH were assessed as well.

RESULTS

TamR BCCs show a significant increase of both basal and maximal respiratory capacity. The expression of a constitutively active FoxO3a counteracts the increased oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) observed in TamR BCCs, causing a reduction of the energetic activity and of the glycolytic rate. Interestingly, active FoxO3a increases mitochondrial biogenesis although reducing mitochondrial functionality by increasing ROS production.
In addition, FoxO3a mitigates the biosynthetic needs of TamR BCCs, as evidenced by a reduction of G6PDH activity (although increased expression) and a decrease in PGLS and PGD expressions, which are involved in the oxidative branch of the pentose phosphate pathway (PPP). Interestingly, an accurate analysis of cBioPortal datasets of BC patients showed a significant correlation of these proteins and FoxO3a.

CONCLUSIONS

FoxO3a decreases glucose catabolism by lowering both the glycolysis and the PPP in TamR cells, which may contribute to overcome tamoxifen resistance. Therefore, therapeutic approaches aimed at reactivating FoxO3a might be helpful in the management of patients with breast tumors refractory to the antiestrogen therapy.

P088

METFORMIN INHIBITS GLIOBLASTOMA CELL PROLIFERATION AND SPREADING BY MODULATION OF SURVIVIN

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BACKGROUND-AIM

Glioblastoma multiforme (GBM) represents the most aggressive subtype of malignant brain tumor. Due to the high invasiveness of the cancer cells, postoperative relapse is very frequent in patient and many GBM possess resistance to radio/chemotherapy which makes the actual standard of care ineffective. Therefore, the development of new therapies and/or therapeutic combinations represents the most important challenge for this neoplasm. In order to highlight additional mechanisms through which the anti-diabetic drug Metformin interferes with GBM growth, we focused our attention on its action on Survivin, a member of the inhibitor of apoptosis (IAP) family and a key factor in GBM cell survival, radio/chemoresistance acquisition and cancer recurrence.

METHODS

Protein expression was evaluated by western blotting (WB) assay; cell viability was analyzed by MTT assay; cell proliferation was investigated by trypan blue exclusion, colony formation and anchorage-independent soft agar assays, cell invasiveness was estimated by invasion assay and by zymography assay evaluating the cellular release of metalloproteases, cell motility was evaluated by transwell migration assay, wound healing assay and phalloidin staining.

RESULTS

Metformin inhibited cell viability, proliferation, as well as migration and invasiveness of U87MG and T98G GBM cell lines. Notably, following Metformin treatment, a lower colony formation capability was observed in each cell lines. These events were paralleled by activation of the AMPK signaling pathways, nuclear translocation of trascriptionl factor FOXO3a and changes in Survivin protein expression which appears to be significantly reduced, unveiling a novel mechanism involved in the anti-cancer activity of Metformin in GBM.

CONCLUSIONS

The anti-diabetic drug Metformin is able to efficiently suppress growth, viability, cell motility and invasiveness in different GBM cell lines through a molecular mechanism involving activation of AMPK/FOXO3a/Survivin pathway. Althrough still preliminary, these results could open new avenues for a future potential use of Metformin in the adjuvant therapy of GBM.

P089

LIVE OR HEAT-KILLED PROBIOTIC AEROSOLIZATION DECREASES ADENOMATOUS LUNG CANCER DEVELOPMENT IN A MOUSE CARCINOGEN-INDUCED TUMOR MODEL

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BACKGROUND-AIM

Lung cancer is a leading cause of cancer death; 85% of cases is related to smoke and environmental carcinogens, that act as tumor initiators/promoters. Screening by computed tomography in high risk population, as smokers, detects at risk nodules and early-stage disease, but, even after resection, recurrence rate is high. The establishment of an immunosuppressive environment strongly favours pre-malignant lesions progression and recurrence.
Lung microbiota contribute to immunosuppression. Here, we explore if antibiotics or probiotic aerosol, that perturb the local microbiota, interferes with lung cancer growth in a mouse carcinogen-induced tumor model.

METHODS

4 weeks A/J and BALB/c mice were injected with urethane (1g/kg) and aerosolized with vancomycin/neomycin (50/100 mg) (V/N) or L. rhamnosus GG (alive or heat killed; 5×109 units) (L. RGG) by a tower inhalation system (Emms) for 16 weeks. Gene expression profile was assessed by Affymetrix Clariom S Pico assay and IHC used to validate the results.

RESULTS

In A/J mice, the number, area and diameter of tumor nodules were reduced by live and heat-killed L. RGG aerosol (p#0.05), while V/N only decreased nodules diameter. A trend suggesting reduction of nodules by these agents was also observed in BALB/c mice, that have a lower sensitivity to the carcinogen. Transcriptional profile of A/J lungs revealed modulation of genes at FDR < 0.05 only in probiotics-treated mice, among which we detected JChain gene, encoding the joining chain of secreted immunoglobulins, enriched both in heat killed- and live L. RGG-treatments. In heat-killed L. RGG- group down modulation of Git1, a G protein-coupled receptor involved in cell migration, and of genes of Git1-related pathways were observed. TIMER 2.0 analysis predicted a significant increase of B, NK and Mast cells, monocytes, granulocytes and neutrophils in L.RGG-treated lungs, while V/N only increased granulocytes/neutrophils. Interestingly, in silico analysis of lung cancer dataset revealed an association between high level of JChain or Git1 mRNA and good or poor prognosis, respectively, in lung adenocarcinoma patients.

CONCLUSIONS

This study highlights probiotic aerosol efficacy in reducing the growth of adenomatous lung cancer, paving the way to the use of this feasible strategy in high risk individuals.

P090

EFFECTS OF THE INHIBITION OF GLUCOSYLCERAMIDE SYNTHASE IN NSCLC CELL LINES

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BACKGROUND-AIM

Osimertinib is a third-generation mutant-selective EGFR tyrosine kinase inhibitor approved for the treatment of EGFR-mutated Non-Small Cell Lung Cancer (NSCLC) patients. Despite the efficacy of osimertinib, the development of resistance eventually occurs. The mechanisms of resistance are heterogeneous and not fully understood, and their characterization is essential to find new therapeutic strategies to delay or overcome osimertinib-resistance.

Ceramide, a well-known regulator of apoptosis, is converted by glucosylceramide synthase (GCS) in Glucosylceramide (GlcCer). A higher content of GlcCer was observed in malignant lung pleural effusions from NSCLC patients and their role in drug-resistance has been well documented in many tumor cells.

METHODS

Experiments were performed in PC9 and PC9T790M osimertinib-sensitive (OS) EGFR-mutant NSCLC cell lines and in their derived osimertinib-resistant clones (OR). Cell lipidome was profiled by UHPLC coupled to high-resolution mass spectrometry with ion-mobility separation. Cell proliferation, cell death, colony formation and signaling transduction pathways have been evaluated.

RESULTS

OR cells showed an increased intracellular content of (poli)glicosylceramides in comparison with OS cells. Inhibition of GCS by PDMP caused inhibition of cell proliferation and colony formation, induction of apoptosis and inactivation of AKT and ERK1/2 in OR cells. PDMP inhibited cell proliferation also in the OS cells, but did not affect the viability of normal cells. In OS cells the combination of PDMP with osimertinib enhanced the inhibition of proliferation and the induction of cell death compared to single treatments. Very interestingly, in OS cells the addition of PDMP significantly prevented the onset of resistance to osimertinib in long-term experiments, through eradication of the persistent drug-tolerant cell subpopulation.

CONCLUSIONS

Our results indicate that the dysregulation of ceramide metabolism may play a role in the mechanisms of resistance to osimertinib. In particular, our data suggest that GCS targeting may be a promising therapeutic strategy to treat EGFR-mutant NSCLC patients progressed to osimertinib and the inhibition of GCS combined with osimertinib may prevent osimertinib-resistance in first-line treatment.

References:

La Monica S Cancers 2020
Eltayeb K Cells 2021
Yong-Yu L Cancer Res. 2013
Stefanovic M Oncotarget 2016

P091

INVESTIGATION OF FUNCTIONAL ASSOCIATIONS BETWEEN THE ESTROGEN RECEPTOR ALPHA AND ESSENTIAL LNCRNAS IN HORMONE-RESPONSIVE BREAST CANCER.

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BACKGROUND-AIM

Breast cancer (BC) is one of the most frequently diagnosed malignancies in women, with a high mortality rate worldwide, due to molecular heterogeneity and resistance to current therapies causing disease recurrence. Estrogen receptor alpha (ERα), a ligand inducible nuclear receptor and transcriptional regulator, plays an important role in clinical management of breast cancer patients, being the key factor in mitogenic estrogen signaling and representing the target of the endocrine-based therapies. Recently, we and others identified a new function of ERα as RNA binding protein (RBP), revealing a new class of ERα functional partners and pointing to the receptor as a multifaceted modulator of gene expression controlling BC progression.

METHODS

With the aim to identify RNA molecules acting in concert with ERα in the formation of multi-molecular regulatory complexes in BC cell genome, native nuclear RNA immunoprecipitation coupled to next generation sequencing (RIP-Seq) was performed in exponentially growing MCF-7 cells. Functional assays to evaluate cell proliferation and apoptosis were performed after silencing selected ERα-binding RNAs.

RESULTS

RIP-Seq generated a dataset of about 3.000 ERα-interacting transcripts, including 166 lncRNAs. This class of noncoding RNAs was selected for further investigation due to their key role in nuclear processes, in particular transcriptional regulation and epigenetic chromatin modifications. Among ERα-bound lncRNAs, we focused on 3 molecules overexpressed in hormone-responsive breast cancer cells and previously shown to be involved in fundamental functional pathways in cancer cells. Indeed, their silencing strongly affected BC cell proliferation and survival in different ways, including modulation of ERα expression.

CONCLUSIONS

The lncRNAs identified and characterized here represent new functional components of the estrogen signaling in luminal-like BC cells and potential targets for novel therapeutic approaches aiming at disrupting estrogen signaling and overcoming resistance to current endocrine therapies.

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P092

DEVELOPMENTAL EXPRESSION OF RECEPTORS FOR ANTI-MÜLLERIAN HORMONE, GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR 1 IN IMMATURE AND MATURE GNRH NEURONAL CELL LINES AND IN THE MOUSE BRAIN

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BACKGROUND-AIM

Gonadotropin-releasing hormone (GnRH) neurons are key neuroendocrine cells in the brain, since they control reproduction by driving the hypothalamic-pituitary-gonadal axis (Oleari et al., 2019). In this context, anti-Müllerian hormone (AMH), growth hormone (GH), and insulin-like growth factor 1 (IGF1) systems were shown to participate to development and function of GnRH-secreting neurons (Passarelli et al., 2022) and when defective to play a role in hypogonadotropic hypogonadism pathogenesis (Cannarella et al., 2021).

METHODS

The expression of receptors for AMH (AMHR2), GH (GHR) and IGF1 (IGF1R) was analyzed by immunofluorescence in in vitro and ex vivo mouse models. Established models of immature (GN11) and mature (GT1-7) GnRH neuronal cell lines and mouse brain sections at different developmental stages were used. Specifically, at E14.5 we focused on vomeronasal organ (VNO) and nasal forebrain junction (NFJ), two regions where GnRH neurons originate and migrate en route to the hypothalamus, respectively. At E18.5 we looked at the median eminence, the area in the hypothalamus where GnRH is released.

RESULTS

In both GN11 and GN1-7 cells, AMHR2 showed a diffuse signal all over the cytoplasm, whereas GHR revealed a clear signal, localized around the nucleolus and on the plasma membrane lamellipodia. IGF1R was weakly expressed only in the cytoplasm of GT1-7 cells. At E14.5, double staining for the neuronal marker beta-tubulin III and AMHR2, GHR or IGF1R revealed signal at the level of VNO and NFJ. Instead, none of these receptors was co-expressed with GnRH. At E18.5, no receptor expression was detected in GnRH neurons.

CONCLUSIONS

GHR expression on the tips of plasma membrane in both cell lines may suggest a role in cell-to-cell communication, and maybe in migration itself. Ex-vivo experiments show AMHR2, GHR and IGF1R expression at E14.5 in the VNO and NFJ, whereas, at E18.5, no expression was detected in the median eminence, highlighting a diminishing expression through the migration process. In addition, GHR and IGF1R localization close to GnRH neurons at E14.5 could be indicative of a supporting role in GnRH neuron migration.

P093

REGULATION OF INSULIN SECRETION BY DOPAMINE

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BACKGROUND-AIM

Pancreatic beta-cells respond to dopamine (DA) and are equipped with a complete dopaminergic apparatus. Different human and rodent beta-cell lines express DA receptors (D1-D5) and enzymes for DA production and degradation. In beta-cells, DA is stored in insulin granules via vesicular monoamine transporter-2 (VMAT-2) and co-released with insulin. The aim of the present research has been to extensively investigate the role of DA in the regulation of insulin secretion by beta-cells.

METHODS

Experiments were performed on the INS-1 832/13 beta-cell line derived from the parental cell line INS-1 by the stable transfection with a plasmid containing the human pro-insulin gene. Insulin secretion was assayed after stimulation with glucose (11 mM), isobutyl-methylxanthine (IBMX, 1 mM) or KCl (30 mM) in the presence or absence of 10 mM DA. The intracellular localization of the main DA receptor (D2) was investigated by immunogold labelling and electron microscopy observation. The glucose-induced co-release of insulin, DA and D2 was investigated by immunofluorescence microscopy. Finally, the effect of VMAT-2 inhibition by tetrabenazine (TBZ) on ROS production and GSH intracellular levels was assayed.

RESULTS

Our results clearly show that DA significantly inhibits insulin secretion stimulated by all the utilized secretagogues, while basal secretion is not modified. This inhibitory effect requires the presence of DA during the incubation with the selected secretagogue. Immunogold labelling demonstrated that D2 receptor is localized inside the insulin granules and that its intragranular concentration decreases upon glucose stimulation. Co-release of insulin, DA and D2 upon glucose stimulation was also confirmed by immunofluorescence observations. Blocking the entry of DA into insulin granules by the VMAT-2 inhibitor TBZ causes a significant increase of intracellular ROS production and GSSG/GSH ratio, probably due to cytoplasmic DA degradation by monoamine oxidases.

CONCLUSIONS

Our results demonstrate that dopamine plays a key role in the regulation of insulin secretion by pancreatic beta-cells. This effect could be related to the observed epidemiological association between Parkinson disease and type 2 diabetes.

P094

AN UPDATED ANALYSIS OF BRCA1 AND BRCA2 GENE MUTATIONS IN BREAST CANCER PATIENTS FROM EASTERN SICILY

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BACKGROUND-AIM

Germline mutations of BRCA1 and BRCA2 are associated with a defined lifetime risk of breast (BC), ovarian (OC) and other cancers. Testing BRCA genes is pivotal to assess individual risk, but also to pursue preventive approaches in healthy carriers and tailored treatments in tumor patients. In fact, the introduction of target BRCA therapies in clinical practice, has allowed personalized medicine in preselected patient cohorts. Aim of our study was to investigate the incidence and distribution of BRCA pathogenic germline alterations in a cohort of BC patients from eastern Sicily and to evaluate their associations with specific BC features.

METHODS

Mutational status of BRCA was assessed in a cohort of 553 BC patients. Genomic DNA was extracted from peripheral blood samples collected in EDTA tubes. The “Oncomine™ BRCA Research Assay” panel was used to perform Next generation Sequencing (NGS). BRCA pathogenetic mutations, validated by Sanger Sequencing, were correlated with tumor grading and proliferation index.

RESULTS

Overall, 46 patients (8.3%) harbored a BRCA pathogenic variant, 23 (50%) in BRCA1 and 23 (50%) in BRCA2. BRCA1 alterations were prevalent among triple negative BC patients, whereas BRCA2 mutations were more common in subjects with luminal B like BC. Tumor grading and proliferation index were both significantly higher among subjects with BRCA1 variants compared to non-carriers.

CONCLUSIONS

Our findings provide an overview about BRCA mutational status among BC patients from eastern Sicily and confirm the role of NGS analysis to identify hereditary BC patients. Overall, these data are consistent with previous evidences supporting BRCA screening to properly prevent and treat cancer among mutation carriers.

P095

DISCOVERY OF NOVEL HUMAN LACTATE DEHYDROGENASE INHIBITORS: STRUCTURE-BASED VIRTUAL SCREENING STUDIES AND BIOLOGICAL ASSESSMENT

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BACKGROUND-AIM

Cancer cells promote their proliferation and survival by increasing the uptake of glucose and converting it into lactate regardless to the presence of extracellular oxygen. This phenomenon is known as aerobic glycolisis. Since the aerobic conversion of pyruvate to lactate is a primary hallmark of cancer and is catalyzed by lactate dehydrogenase (LDH), we searched for novel inhibitors of this enzyme, as potential new anticancer tools. As a model, we used medulloblastoma, 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 analyzed 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 LDH inhibitor, compound #18, with a robust and specific anticancer activity that was lost in cells lacking LDHA. To strenghten this evidence, we performed LDHA activity assays upon #18 treatment and we observed a significant inhibitory effect. To determine if the inhibition of cell proliferation was due to death or autophagic mechanisms, we analyzed PARP cleavage and LC3B I/II levels by western blot, but both did not significantly change, so the drug alone did not affect it. Conversely, when cells were exposed to #18 and Rotenone, a complex I inhibitor, proliferation was suppressed and programmed cell death was activated.

CONCLUSIONS

Our data support the conclusion that #18 deserves to be further investigated as a starting point for the development of LDH inhibitors and for novel anticancer strategies based on the targeting of key metabolic steps.

P096

POST-TRANSLATIONAL MODIFICATIONS OF NOTCH3 AS A MODUS OPERANDI TO REGULATE ITS PROTEIN ACTIVITY AND STABILITY.

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BACKGROUND-AIM

Notch signaling is a conserved pathway whose deregulation has been implicated in the development of several diseases, including cancer. In particular, it has emerged as a promising candidate for innovative target therapies. Notably, a growing body of evidence supports the notion of Post-Translational Modifications (PTMs) as a modus operandi controlling Notch activity. Indeed, PTMs play a pivotal role in regulating protein function as they modulate protein activity and stability. Therefore, the exploitation of Notch receptors PTMs is emerging as a novel therapy approach, that allows the prediction of the interaction between Notch and other proteins. In this field, the peptidyl-prolyl cis/trans isomerase Pin1 is able to induce PTMs on its substrate recognizing and binding specific phosphorylated residues. In this scenario, we previously demonstrated that Pin1 positively regulates Notch3 (N3) protein expression in T-cell acute lymphoblastic leukemia (T-ALL) aggressiveness and progression and we wondered whether and how N3-Pin1 cross-talk might occur also in ovarian cancer (OC) context. The main aim of this study is the dissection of key molecular oncogenic mechanisms involved in Pin1/N3 cross-talk which may impinge on tumor progression in OC context.

METHODS

Exogenous systems (HEK293T and HEK293T-Pin1KO cells) and endogeous systems (OVCAR3 and SKOV3 OC cells); Transfection; Co-immunoprecipitation assay; Mass-spectometry (MS) analysis; Insertional mutagenesis.

RESULTS

Firstly, we evaluated which amminoacids are responsible for the interaction between Pin1 and N3 by MS and protein conformational analysis. Moreover, we documented that Pin1 sustains the expression of N3 intracellular domain by preventing its proteasomal degradation, promoted by the GSK3β kinase. Finally, we investigated the potential relevance of the Pin1/N3 cross-talk in allowing the escape from this negative regulation process in OC context, through the evaluation of the N3 protein stability and ubiquitination status.

CONCLUSIONS

Our findings showed the presence of two regulators of N3 protein expression, acting one as a positive regulator (Pin1) and the other one as a negative regulator (GSK3β), suggesting an antagonistic effect that affects the N3 stability.

P097

MOLECULAR DISSECTION OF GSK3β-WWP2 CROSSTALK TO DOWNREGULATE NOTCH3 ACTIVITY IN OVARIAN CANCER

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BACKGROUND-AIM

Ovarian cancer (OC) is the fifth leading cause of female cancer-related deaths. Curative and survival trends have not changed significantly because of lack of a definitive screening tool and clear symptoms to prevent early diagnosis. In this scenario, the identification and characterization of targets for novel therapies are urgently required. It is known that Notch signaling pathway has a crucial role in OC. Notably, over-expression of Notch3 (N3) receptor, among the four Notch receptors, has been detected in a wide panel of OC. Therefore, N3-specific inhibition may represent a promising therapeutic strategy for OC cancer treatment. The main aims of this work are: 1. to investigate the molecular mechanism responsible for E3-ligase WWP2-dependent negative regulation of N3-intracelluler domain (N3ICD) and 2. to analyze the crosstalk between WWP2 and the GSK-3β kinase, the last already identified in our preliminary studies as a N3-negative regulator in OC context.

METHODS

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

RESULTS

Firstly, we assessed the interaction between N3ICD and WWP2 proteins by bi-directional co-immunoprecipitation assay. Furthermore, we identified other N3ICD domain, in addition to N3ICD domain containing WWP2-degron site, involved in the WWP2-N3 interaction. Additionally, we evaluated the functional activity of WWP2 upon N3ICD, proving that WWP2 is able to induce the poly-ubiquitination of N3ICD, thus sustaining its involvement in N3ICD degradation. Therefore, we tested if the presence of WWP2 could amplify the negative regulation of GSK-3β on N3ICD. We documented that GSK-3β and WWP2 induced higher decrease of N3ICD protein expression with respect to the presence of the GSK-3β alone. In addition, we also demonstrated that the presence of GSK-3β increased the interaction between WWP2 and N3ICD proteins. Taken together, these findings strongly supported that the E3-ligase WWP2 is implicated in the GSK-3β-dependent proteasomal degradation of N3ICD.

CONCLUSIONS

Overall, our data will help us to identify and validate new molecular targets involved in the negative regulation of N3 oncogenic pathway, finally to develop novel therapeutic strategies.

P098

TARGETING POLYAMINES/EIF5A /MYC TRANSLATION STRONGLY REDUCE COLORECTAL CANCER GROWTH IN PRECLINICAL MOUSE MODELS

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BACKGROUND-AIM

Colorectal cancer (CRC) is the second neoplasia worldwide which causes a high number of deaths every year. The current therapeutic protocols for CRC include only a few drugs with limited efficacy especially in the advanced stages. Then, lots of efforts are aimed at the identification of novel druggable molecular mechanisms in this disease, to unveil new possible therapeutic targets. Polyamine pathway is a promising druggable target in preclinical and clinical models of CRC. Polyamines are policationic molecules with a key biological role in transcription, translation, and autophagy. Several tumors, CRC included, show intracellular elevated content of polyamines compared to normal cells, which could be targeted by difluoromethylornithine (DFMO). Moreover, we demonstrated that polyamine/eIF5A-Hyp/MYC axis could be effectively inhibited through the N1-guanyl-1,7-diaminoheptane (GC7), leading to reduction of cancer growth by blocking MYC elongation. We wonder whether DFMO and GC7 could synergize, leading to a strongest reduction of the CRC cell growth.

METHODS

We performed proliferation assays, growth curve and MTT to test the effects of the individual drugs or the combination on cell proliferation. Gene expression analysis, polysomal fractionation to identify MYC as a target of polyamine/hyp-eIF5A. Cell death studies included JC1-dye incorporation, to tested mitochondrial cell depolarization. We used lentiviral knockdown to silence the enzymes involved in the drug response, and to demonstrate the specificity of the drugs. We analyze the in vivo effect of the drugs administration both in xenograft experiments and in preclinical model of FAP (Familial Adenomatous Polyposis).

RESULTS

We found that MYC is a translational target of Hyp-eIF5A and it is regulated by polyamine/hypusine axis. We show that GC7 is effective in reducing CRC growth and that the combined treatment DFMO and GC7, strongly reduce cell proliferation compared to the administration of the drugs separately, leading to apoptosis. We obtained the same results after selective knock down of the two enzymes DHPS (inhibited by GC7) and ODC (inhibited by DFMO). Finally, we demonstrate in xenograft studies the synergy of the combination treatments.

CONCLUSIONS

Collectively, our data suggest that polyamine/hypusine/MYC axis is a powerful druggable target to impair CRC growth. We conclude that DFMO and GC7 in combination is strongest therapeutic approach to reduce colorectal cancer growth, more than the administration of the drugs separately.

P099

IL-6 AND TNFα LEVELS AND MIRNAS MODULATION IN PERIODONTAL DISEASE

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BACKGROUND-AIM

Periodontitis is one of the most frequent intraoral diseases. Pathogenesis triggers are the immune response with the production of pro-inflammatory cytokines and the non-coding RNAs expression under epigenetic control. The study aimed to evaluate gingival crevicular fluid (GCF) microRNAs (miRNAs) involvement in periodontitis, and their relationship with the main inflammatory mediators' levels and disease stages.

METHODS

21 periodontal disease patients and 15 healthy controls (HC) were enrolled in the study. GCF samples were collected from single-rooted teeth from each subject and used for miRNAs expression analysis using a Real-Time PCR and inflammatory parameters assessment by enzyme-linked immunosorbent (ELISA) assay.

RESULTS

A significant increase in the relative quantification levels of miR-103a-3p, miR-423-5p, miR-23a-3p, miR-15a-5p, and miR-223-3p with respect to HC, was observed. Also, inflammatory mediators evaluated in GCF correlate well with the clinical parameters and with the severity of the periodontal disease, with differences in comparison with HC. A correlation analysis revealed that miR-103a-3p and miR-423-5p have a positive correlation towards TNFa, IL-6 and the disease stage 4, while miR-23a-3p showed a negative trend, suggesting a different involvement in the disease regulation.

CONCLUSIONS

miRNAs as new biomarkers of periodontal diseases may give us more information about the severity and help treatment planning and disease monitoring.

P100

BIOMOLECULAR CHARACTERIZATION OF SW 872 ADIPOCYTE CELLS: AN EXPERIMENTAL MODEL OF HUMAN ADIPOCYTES

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BACKGROUND-AIM

A limited number of human adipocyte cell models is currently available for in vitro adipose tissue studies, suggesting the need for a novel well-characterized human adipocyte model, which would contribute to explore the pathophysiology of obesity and related cardio-metabolic complications. To begin to address this issue, we aimed to characterize the adipocyte-related molecular features of the SW 872 human liposarcoma cell line, previously used mostly for experimental oncology studies, and assessed its usefulness to evaluate the effects of natural bioactive compounds with potential health properties.

METHODS

SW 872 cells were studied under non-differentiated and differentiated conditions (7-day exposure to 100 μM oleic acid (OA)). Triglyceride (TG) accumulation was evaluated by oil-red-O and TG quantification; the modulation of Akt protein phosphorylation (pAkt) by Western Blot, glucose uptake by FACS analysis; and pro-inflammatory cytokine release by ELISA. We then analysed the effect of one herbal extract on cytotoxicity by MTT assay, and on glucose uptake by FACS.

RESULTS

SW 872 cells that were differentiated with OA showed a higher TG content ($p < 0.001$) compared to non-differentiated cells, a lower glucose uptake ($p < 0.001$), and a peculiar activation of the Akt pathway. Additionally, we observed a time-dependent modulation of the basal secretion of pro-inflammatory cytokines, with an increase of interleukin-6 and a reduction of interleukin-8, after treatment with OA ($p < 0.01$ and $p < 0.001$, respectively).
The extract did not show cytotoxicity after 12, 24 and 48 h of incubation, and stimulated glucose uptake in a dose dependent manner. Specifically, a 41.01% increase was observed at the concentration of 0.5 mg/mL ($p < 0.01$) and an increase by 63.96%, in 1 mg/mL ($p < 0.01$) concentration, both after 24 h of treatment.

CONCLUSIONS

These findings suggest that SW 872 cells may represent a reliable adipocyte model of human origin, useful for a better understanding of some molecular features of the pathophysiology of obesity and cardiometabolic diseases.

P101

BIOMARKERS OF CELL AND NEURONAL DAMAGE AND IMMUNE ACTIVATION ASSOCIATED WITH INTRA-HOSPITAL MORTALITY IN GERIATRIC COVID-19 PATIENTS

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BACKGROUND-AIM

The severity of COVID-19 shows significant variation, from mild illness to severe disease requiring hospitalization and, in the most critical cases, leading to death. The analysis of circulating biomolecules could lead to the discovery of new biomarkers of COVID-19 severity, aimed to improve the clinical management of subjects at higher risk of severe outcomes, especially in the setting of geriatric patients.

METHODS

In a cohort of COVID-19 geriatric patients who required hospitalization we analysed, on-admission, a series of circulating biomarkers related to neutrophil activation (neutrophil elastase, LL-37), macrophage activation (sCD163), cell damages (nuclear cfDNA, mitochondrial cfDNA, and nuclear cfDNA integrity) and neuronal damage (neurofilament-light chain (NF-L)). The above-reported biomarkers were tested for their association with intra-hospital mortality and clinical, inflammatory, and routine hematological parameters.

RESULTS

Lower n-cfDNA integrity, higher neutrophil elastase, and higher sCD163 levels were significantly associated with an increased risk of intra-hospital decease. Median (IQR) values observed in discharged vs. deceased patients were: 0.5 (0.3-0.7) vs. 0.3 (0.2-0.6) for n-cfDNA integrity; 94.0 (47.7-154.0) ng/ml vs. 115.7 (84.2-212.7) ng/ml for neutrophil elastase; 614.0 (370.0-821.0) ng/ml vs. 787.0 (560.0-1304.0) ng/ml for sCD163. The analysis of survival curves in patients stratified for tertiles of each biomarker showed that patients with n-cfDNA integrity < 0.32 or sCD163 in the range 492-811 ng/ml had a higher risk of intra-hospital decease than, respectively, patients with higher n-cfDNA integrity or lower sCD163. Moreover, an association between NF-L and intra-hospital mortality was found. Notably, among the tested biomarkers, plasma neutrophil elastase, n-cfDNA, and mt-cfDNA resulted to be significantly more abundant in patients showing SARS-CoV-2 RNAemia on-admission than in patients who resulted negative for this parameter.

CONCLUSIONS

On the whole, biomarkers of cell-free DNA integrity, immune activation, and neurodegeneration contribute to identifying geriatric patients with a high risk of COVID-19 intra-hospital mortality.

P102

IDENTIFICATION OF A CD117+ CD 133- NEURAL STEM CELL POPULATION ENRICHED IN NEURONAL PRECURSOR.

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BACKGROUND-AIM

Neurodegenerative diseases (NDs) affect millions of people worldwide. Combination of genes, aging and environment contributes to the risk of developing these diseases. Although certain treatments may help to relieve some symptoms, currently no cures exist.
Recently, transplantation of neural stem cells (NSCs), has been investigated as a new therapeutic approach. However, it is challenging to characterize the most efficient NSCs population that retains positive effects after transplantation. In the adult mammalian brain, a population of self-renewing multipotent cells has been identified in specific regions: subgranular zone, the subventricular zone, hypothalamus and the olfactory bulb. This population retains the potential to generate cells representing the main phenotype of the nervous system: neuron-astrocyte-oligodendrocyte.
Identification of NSCs is hampered by the lack of specific markers, many proteins are found to be expressed in neural progenitor cells, but most of them are not exclusive and importantly, are intracellular markers not usefull for prospective stem cell isolation. Prominin 1 (CD133) and or CD15 were successfully used to isolated neural precursor cells that are clonogenic and tripotent. However, these cell surface markers where invariably expressed heterogeneously in proliferating cultures.
CD117 (KIT) is a tyrosine-protein kinase that acts as cell-surface receptor for the cytokine KITLG/SCF and plays an essential role in the regulation of stem cell maintenance, hematopoiesis, gametogenesis. It has also been demonstrated that CD117 is a marker for adult stem cell population in different organs.

METHODS

Adult mice brain sections and in FACS sorted CD117+ CD133- cells compared to CD133+ cells, isolated from hippocampus and cultured as neurosphere were used.

RESULTS

We identified a rare population (0.37%) expressing Kit in adult hippocampus, that retains the capability to generate neurosphere for many passages and to differentiate preferentially into neurons. They also express a combination of NSC markers including Nestin, Sox2, DCX and GFAP cells; most of them were lineage and tryptase negative. Furthermore, Kit-labeled cells became NeuN+ and betaIII-Tubulin positive suggesting a neuronal differentiation.

CONCLUSIONS

Further studies will be conducted to better characterized this newly identified NSCpopulation

P103

IS THERE A TOXIC IMPACT OF COMMERCIAL RUSSIAN CHRYSOTILE ON HUMAN HEALTH? AN IN VITRO STUDY ON HUMAN LUNG CELLS AS A TARGET OF INHALED ASBESTOS FIBRES

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BACKGROUND-AIM

In the last 50 years, research on asbestos minerals have been intensively conducted to evaluate their toxicity/ pathogenicity and effects on human health. Among asbestos fibres, chrysotile or "white asbestos", a hydrated magnesium layer silicate, due to its outstanding technological properties is the most intensively used fibre in industry, despite is classified by the International Agency for Research on Cancer as "carcinogenic to humans (Group 1)" and is known to induce lung diseases such as asbestosis, lung cancer and mesothelioma. Russia is the largest chrysotile producer in the world where it is still used in "safe mode".

METHODS

Russian chrysotile fibres with different length: R1 <5μm and R2 >5μm were administered in cultured mesothelial (MeT5A) and alveolar (A549) human cells lines. UICC crocidolite and NYAG wollastonite fibres were considered as positive and negative controls, respectively. MTT assay and microscopy investigations were performed to evaluate the cytotoxicity effect. Reactive Oxidative Species (ROS) production, depletion of Glutathione levels and expression of pro-inflammatory cytokines by BioPlex were evaluated to investigate the oxidative status. Comet assay and study of cell cycle were performed to assess chrysotile genotoxic effect. miRNA-126 and miRNA-222 were evaluated as miRNAs related to mesothelioma pathway

RESULTS

Different treatment responses were found with a major sensibility in MeT5A compared to A549 cells. High cytotoxic effects exerted by asbestos fibres were observed in both cell lines showing decrease of cell viability and high cell vacuolization. On the basis of cytotoxic effect, a great production of ROS and decrease of glutathione levels were sustained by overexpression of pro-inflammatory cytokines. This observation was in line with the high grade of DNA damage correlated with a block in G2/M phase of cell cycle. The early upregulation (6h) of miRNA-126 and -222 in both cell lines suggested the alteration of typical target genes involved in cancer progression, promoting the angiogenesis and the macrophages induction

CONCLUSIONS

This study aims to mimic the cyto- and geno-toxic impact of Russian chrysotile fibres in lung human tissue. Our data support the high potential risk of long chrysotile fibre compared to the short ones with a paramount role played by frustrated phagocytosis.

P104

SUPPRESSION OF BDNF-TRKB.T1 SIGNALING IN MICE HEART EXACERBATES THE CARDIOTOXIC EFFECT OF DOXORUBICIN LEADING TO HEART FAILURE.

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BACKGROUND-AIM

Doxorubicin (DOX) is an anthracycline antibiotic with antineoplastic activity highly effective for the treatment of many tumors. Per contra, it often causes cardiotoxicity that can be chronic taking place months or even years after DOX-treatment. There is a high risk to develop cardiomyopathy after the end of chemotherapy. Despite the several studies conducted, the biological mechanism underlying DOX cardiotoxicity is not yet fully understood and importantly the risk for DOX cardiotoxicity remains unpredictable.

Recently we proved that BDNF-TrkB.T1 signaling is important in mouse heart: removal of BDNF or its truncated receptor TrkB.T1 induce dilated cardiomyopathy. We also showed that BDNF acting on this isoform elicits calcium sparks in isolated adult cardiomyocytes, calcium handling and homeostasis seems to be a central target of BDNF-TrkB.T1 signaling in non-neuronal cells type.

Calcium handling and homeostasis is involved in some of the proposed mechanisms of DOX cardiotoxicity such as ROS production and oxidative stress, mitochondria dysfunction, sarcoplasmic reticulum function and cell death. Therefore, it seems reasonable that BDNF-TrkB.T1 signaling may have a role in the pathogenesis of DOX cardiotoxicity.

METHODS

We generated the TrkB.T1 mouse model and tested if the ablation of BDNF-TrkB.T1 signaling in the cardiac tissue may alter the onset and progression of DOX induced cardiomyopathy. In mice treated with Doxorubicin (I.P. for five consecutive weeks up to a cumulative dose of 25 mg /kg body weight), we measured cardiac performances using ex-vivo Langendorff preparation and evaluate cardiac morphological alteration by light and electron microscopy.

RESULTS

We found that the severity of cardiomyopathy, in DOX treated TrkB.T1 knockout mice, dramatically increased as shown from the morphology, fibrotic area and necrotic fibers were detected; reduction of developed pressure more evident under dobutamine stimulation, was also measured. Furthermore, we tested the TrkB isoform and BDNF expression in the human heart, we found that it closely resembles the mouse heart expression pattern.

CONCLUSIONS

Considering this new evidence, it is founded to suggest that the BDNF-TrkB.T1 signalling may be a valuable target to be studied for possible pharmacological intervention to limit the cardiac damage induced by anthracyclines treatment.

P105

PROFILING OF IMMUNE-RELATED BIOMARKERS IN MALE AND FEMALE BRCA-ASSOCIATED BREAST CANCERS

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BACKGROUND-AIM

Germline BRCA1 and BRCA2 (BRCA) pathogenic variants (PVs) are associated with increased breast cancer (BC) risk in both sexes, and represent crucial predictive biomarkers for targeted therapy with PARP inhibitors and immunotherapy. However, genetic and gender-related differences in immune determinants may contribute to diverse therapeutic outcome in male and female patients.

We investigated established immune-related biomarkers in BCs, in relation with BRCA status and gender, with the aim to provide key insights that may help improving the selection of patients responsive to immunotherapy.

METHODS

A total of 53 BRCA-BCs, including 10 BRCA1 and 10 BRCA2 female BCs (FBCs) from The Cancer Genome Atlas, and 12 BRCA1 and 21 BRCA2 male BCs (MBCs) from our Italian Multicenter Study on MBC, were analyzed. From the same sources, data on non-BRCA BCs (1,080 FBCs and 74 MBCs) were included for comparison. Tumor mutational burden (TMB), PD-1 and PD-L1 expression, immune scores and immune cell infiltration profiling by ESTIMATE and CIBERSORT were computed from genomic and transcriptomic data.

RESULTS

Similar to BRCA-FBCs, BRCA-MBCs showed higher TMB values compared with non-BRCA MBCs ($p=0.03$), but comparable PD-1/ PD-L1 expression and immune score levels.

In male and female patients, BRCA-BCs showed comparable immune score levels. Notably, BRCA-FBCs had higher PD-1 expression levels compared with BRCA-MBCs ($p=0.0002$).

BRCA-MBCs and BRCA-FBCs showed statistically significant ($p=<0.05$) differences in the fractions of several tumor infiltrating immune cells: M1 Macrophages, Gamma-Delta T cells, Plasma cells, resting Dendritic cells and Neutrophils were more abundant in BRCA-FBCs; CD4+ memory resting T cells, Monocytes, activated NK cells and Eosinophils were more abundant in BRCA-MBCs.

When considering the two genes separately, the fraction of M2 Macrophages was significantly higher in BRCA1-FBCs compared with BRCA1-MBCs ($p=0.0002$), and in BRCA2 compared with BRCA1 BCs ($p=0.002$) in men only.

CONCLUSIONS

Our analysis highlighted relevant differences in immune-related biomarkers of breast tumors associated with BRCA PVs and gender. These results may add data to further elucidate the determinants of response to immunotherapy.

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P106

IDENTIFICATION OF MAML1 AS A NOVEL NEGATIVE REGULATOR OF ITCH E3 UBIQUITIN LIGASE ACTIVITY: NEW INSIGHTS IN CANCER BIOLOGY

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BACKGROUND-AIM

In mammals, Maml1 act as transcriptional coactivators for Notch signalling, an evolutionarily conserved pathway. Maml1 has been recently shown to act as coactivator in other signalling pathways, such as p53, Wnt and Hippo in a Notch-independent manner. More recently, we have demonstrated the involvement of Maml1 in the Hedgehog pathway, behaving as a novel co-activator of Gli1/2 transcription factors. Hedgehog and Notch signalling pathways are directly involved in the onset/development of several cancers and are both regulated at post-translational level by Itch/E3 ubiquitin ligase protein. Itch activity is enhanced by the adaptor Numb that is able to bind Itch-WW domains to induce ubiquitination and degradation of Gli1 and Notch1.

METHODS

Immunoprecipitation and ubiquitination assays in both in vitro and ex vivo cell lines; Maml1 silencing with CRISPR/Cas9 technology; analysis of Itch post-translational modification; siRNA-mediated depletion of Maml1 in breast and colon cancer cell lines; wound healing assay; proliferation assays.

RESULTS

Here, we demonstrate that Maml1 can control the expression levels of Gli1 and Notch1 at post-translational level, by directly inhibiting Itch catalytic activity. For the first time, we identify the functional role of the Maml1 C-terminal domain as a post-translational regulator of target proteins. Moreover, we pinpoint the molecular mechanism through which Maml1 acts as negative regulator of Itch, by inducing auto-ubiquitination events. Therefore, Maml1 increases the expression levels of Gli1 and Notch1 oncogenic proteins, by switching off Itch activity. Accordingly, in pathological contexts, such as breast and colon cancers, Maml1 silencing impinge on Notch1 and Gli1 protein levels, hindering proliferation and epithelial-mesenchymal transition events.

CONCLUSIONS

Overall, our data suggest a protective role mediated by Maml1 on Itch-target proteins involved in cancer biology. The ability of Maml1 to negatively regulate Itch activity could have an impact in the activation of oncogenic pathways, such as Hedgehog and Notch. The identification of Maml1 as a novel negative regulator of Itch adds a piece in the understanding of tumour biology and could help to set out new therapeutic approaches based on the dual role of Maml1.

P107

IMPROVING GENDER-SPECIFIC BREAST CANCER PRECISION PREVENTION: GENETIC RISK ASSESSMENT USING MULTI-GENE PANEL TESTING IN MALE BREAST CANCER

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BACKGROUND-AIM

Germline pathogenic variants (PVs) in BRCA1/2 genes are associated with high breast cancer (BC) risk in both women and men. Reliable data on actionable PVs in other cancer susceptibility genes are emerging, with relevant implications in terms of BC prevention and treatment. Thus, multigene panel tests are being increasingly used for BC risk assessment, although gender-specific data are lacking. This study aimed to improve BC precision prevention by providing the spectrum, prevalence and risk estimates associated with PV in non-BRCA genes for men with BC.

METHODS

A population-based case-control study, including 725 BRCA1/2 PV tested negative MBCs and 1076 healthy male controls, all enrolled in the frame of the first Italian multicenter study on MBC, was performed using a custom 50-gene panel in NGS. All PV identified were confirmed by Sanger sequencing. Statistical analyses were performed using chi-square test and logistic regression model.

RESULTS

Actionable PVs in cancer susceptibility genes besides BRCA1/2 were identified in 37 out of 725 (5%) MBC cases and in 20 out of 1076 (1.8%) controls, mainly PALB2, ATM, BLM, FANCM and CHEK2. Overall, PVs were significantly more frequent in MBCs compared with controls and were associated with a 3-fold increased MBC risk (5% vs 1.8%, OR: 3.3, 95% CI: 1.7-6.3; p=0.0006).
PALB2 PVs were identified in 1% of MBCs and were associated with a 7-fold increased MBC risk (OR:6.8, 95% CI: 1.1-42.9; p=0.04). BLM and CHEK2 PVs were more frequent in cases than in controls (p=0.02 and p=0.03, respectively).
PV carriers were more likely to have personal (p=0.01) and family (p=0.006) history of cancers not limited to BC.

CONCLUSIONS

Our results highlight the main role of PALB2 PVs in MBC susceptibility and provide cancer risk estimates with possible translational relevance.
This study supports the clinical utility of multigene panel testing approach to increase the detection of actionable PVs in MBC patients and, in particular in those with family and personal history of multiple cancers.
Overall, these results may improve gender-specific BC precision prevention in patients and their relatives.
Study supported by AIRC (IG21389) to LO.

P108

HOST P2X7R DELETION FAVOURS IMMUNOSUPPRESSION AND NEOVASCULARIZATION THROUGH A2AR UPREGULATION DURING ONCOGENESIS

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BACKGROUND-AIM

In tumor microenvironment extracellular ATP and P2X7 receptor (P2X7R) play a central role in cancer growth and progression. ATP and adenosine recently emerged as key constituents of the tumor niche where they exert opposite and complementary roles. ATP promotes tumor growth but also immune eradicating responses mainly via the P2X7R, while adenosine is a potent immune suppressor and facilitates neovascularization thanks to A2A receptor (A2AR) activity. However, although both mechanisms have been investigated in preclinical models, studies exploring the interplay between P2X7R and A2AR in the tumor microenvironment are as yet missing.

METHODS

We examined tumor growth in C57/bl6 P2X7 wild type and null mice inoculated with B16-F10 melanoma cells and analyzed a panel of the main pro-inflammatory and immunosuppressive cytokines (IL1-β, TNF-α, IL-6, IL-12, IL-17, IFN-γ, TGFβ). We also investigated the expression of A2AR in tumors and other organs derived from the same mice, treated or not with SCH-58261, an A2AR antagonist. Finally, we analyzed blood vessel formation by histochemistry and CD31 staining.

RESULTS

We obtained a significant reduction of systemic levels of proinflammatory cytokines and an increase of TGFβ in tumors growing in P2X7R null mice compared to wild type. In the same tumors there is also an upregulation of A2AR and an increase of blood vessels formation, accompanied by a rise in VEGF levels, suggesting that immunosuppression associated to lack of the P2X7R might depend upon A2AR overexpression. Moreover, SCH-58261 administration reduced tumor growth and intramass levels of TGF-β similarly in the P2X7 wild type or null mice strain, thus supporting a key immune suppressive role of A2AR in our model.

CONCLUSIONS

Host P2X7 deletion causes immunosuppression and neovascularization through A2AR upregulation. The inhibition of both receptors may represent a possible pharmacological strategy to promote antitumor immune response and reduce neovascularization in the tumor microenvironment.

P109

β1 INTEGRIN COMPLEX INTERACTION WITH ION CHANNELS UPON FORMATION OF MACROMOLECULAR COMPLEXES ENCOMPASSING LIPID RAFTS TO MODULATE CANCER PROGRESSION.

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BACKGROUND-AIM

hERG1 channels are often aberrantly expressed in human cancers, where they regulate many stages of tumorigenesis. Furthermore, hERG1 and the ß1 subunit of integrin receptors can form macromolecular complexes on the plasma membrane of cancer cells. The latter often comprise integrin adhesion receptors and lipid rafts, which are composed by sphingolipids, including gangliosides and sphingomyelin, cholesterol and signaling proteins and they are part of this traffic. In this study, we combined experimental and theoretical methods to study the relationships between the integrin-dependent hERG1 current activation and the formation of the macromolecular complex between the channel, the ß1 integrin and lipid rafts.

METHODS

We studied the dynamics of hERG1/ß1 integrin complex formation and hERG1 current modulation upon integrin activation by cell adhesion to ECM proteins, using HEK 293 cells transfected with the hERG1 encoding cDNA (HEK-hERG1 cells) as a model. We performed also adhesion, immunofluorescence (IF) and flow cytometry (FACS) experiments. Moreover, the association of hERG1/ß1 Integrin with lipid rafts in HEK 293 hERG1 cells under fibronectin stimulation was investigated.

RESULTS

HEK-hERG1 cells started to adhere 5 minutes after seeding onto Fibronectin (ß1 integrin activator), attaining the plateau value at 90 min. The amount of the hERG1/ß1 integrin complex progressively increased from the time of cell seeding up to 90 minutes of incubation, to decrease rapidly thereafter, reaching very low levels.FACS and IF experiments demonstrated that hERG1 protein had the maximal expression on the plasma membrane at 90 min and before and after this time point hERG1 colocalized with endosomal Rab5 protein. To evaluate the effects of fibronectin on the association of hERG1 with ß1 Integrin into lipid rafts of HEK 293 hERG1 cells, we performed co-ip, showing that the complex is present when cells are stimulated.

CONCLUSIONS

Integrin-dependent cell adhesion triggers the formation of a hERG1/ß1 integrin complex, which progressively increases, with a kinetic similar to an increase in hERG1 current and a hyperpolarization of the membrane resting potential. All these parameters reach their maximum values at 90 min, to decline thereafter. We have shown that such effect is mediated by an increase in hERG1 protein expression. Moreover, the data we have obtained allow us to propose the involvement of lipid rafts in a new molecular hub formed with hERG1/ß1 integrin molecules.

P110

A DEFINITE PANEL OF INFLAMMATORY NEUROLOGICAL MARKERS HELP DIAGNOSIS AND FOLLOW-UP OF ACUTE DEMYELINATING ENCEPHALOMYELITIS.

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BACKGROUND-AIM

Acute demyelinating encephalomyelitis (ADEM) is the most frequent clinical presentation of MOG-associated diseases (MOGAD) in children. In the majority of cases, it is preceded by an infection of the upper respiratory tract. In ADEM, the immune response causes inflammation in the central nervous system, which results in demyelination of nerve fibers. A critical element for diagnosis and follow-up is the detection of MOG autoantibodies (MOG-Ab), but other biological markers, such as inflammatory cytokines and neurofilament light chain (NfL) concentration, may be useful to improve early diagnosis and guide therapeutic intervention in the follow-up.

METHODS

We herein present a paradigmatic case report regarding a 1-year-old female with ADEM, in which we tested MOG-Ab by home-made live-CBA in serum and CSF at diagnosis (oct 2021) and in the follow-up (up to jun 2022). At the same time, we also analyzed a large panel of cytokines and the concentration of neurofilament light chain (NfL) by ultrasentive ELISA.

RESULTS

At baseline, MOG-Ab were present at high titre both in serum and CSF, as well as the NfL levels. Since corticosteroids were not sufficient, the IgEV therapy was started and then, before any successive infusion, we tested MOG-Ab, cytokines and NfL in serum. Before the second IgEV infusion, MOG-Ab and NfL appeared significantly decreased, while both inflammatory (IFNg, CXCL10) and anti-inflammatory (IL10) cytokines were still up-regulated. Before the third infusion, MOG-Ab became negative and both NfL and cytokines significantly decreased. Before the fourth infusion, the MRI reported new demyelinating lesions and MOG-Ab returned at high titre as well as the NfL, while Th1 cytokines remained up-regulated. Before the fifth infusion, the MRI improved, MOG-Ab turned again negative, NfL were significantly reduced, and all cytokines normalized. But before the sixth infusion, MOG-Ab reappeared and also NfL and all cytokines were again up-regulated.

CONCLUSIONS

MOG-Ab appeared significantly correlated to NfL serum levels, demyelination (MRI lesions) and inflammatory cytokines, underlying their fundamental pathogenic role in MOGAD. Some cytokines, IL-2Ra and CXCL10 are particularly correlated with disease behavior and may help to draw new targeted therapeutic strategies in the future.

P111

DISSECTION OF ERK5 NUCLEAR TRANSLOCATION USING A NOVEL APPROACH FOR SINGLE MOLECULE TRACKING

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BACKGROUND-AIM

The extracellular signal-regulated kinase 5 (ERK5) is emerging as a possible target for cancer treatment. ERK5 pro-proliferative activities are linked to its nuclear presence, but the mechanisms involved in ERK5 nuclear translocation are poorly characterized. We focused on the elucidation of this process using single molecule tracking in order to design new strategies for cancer treatment.

METHODS

To achieve single ERK5 tracking in living cells, we used Super-Resolution microscopy. HeLa cells have been transfected with an expression vector for ERK5 linked to HaloTag, alone or with a vector for a constitutively active form of the ERK5 activator MEK5 (MEK5DD). The cell-permeable photoactivatable chromophore JaneliaFluor646, able to recognise Halo Tag, has been used as detection technique. As a complementary approach, HeLa cells, transfected with ERK5 and MEK5DD, have been treated with an importin inhibitor (IMPi). MTT and 2D-colony forming assays were performed in A375 cells treated with IMPi, alone or in combination with the ERK5-i AX-15836.

RESULTS

The HaloTag technology provided the JaneliaFluor646 selective binding to ERK5, and Highly Inclined and Laminated Optical sheet (HILO) microscopy allowed to collect the signal of individual chromophores (i.e. single ERK5 molecules). Obtained data showed that in ERK5-transfected cells the protein is mainly localized in the cytoplasm, whereas it moves in the nucleus with the activator MEK5DD, as expected. This effect was partially reverted in cells treated with IMPi. Accordingly, ERK5 amount in the nuclear fraction of lysates from IMPi-treated-cells was lower compared to control, pointing to the involvement of importins in ERK5 nuclear transport. Finally, we found that ERK5i AX-15836, which has been reported to induce ERK5 nuclear translocation in a paradoxical way, reduced melanoma cell proliferation only in combination with IMPi.

CONCLUSIONS

The HaloTag-JaneliaFluor646 method has proven effective for the localization of single ERK5 molecules with nanometre accuracy, thus providing a novel approach to evaluate how ERK5 moves to the nucleus. The actors involved in these processes could be identify as novel targets for ERK5 inhibition, and therefore for a possible anticancer therapy.

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HIGH BLOOD CONCENTRATION OF CD45-EPCAM+PD-L1- EXTRACELLULAR VESICLES IS ASSOCIATED WITH METASTATIC DISEASE AND POOR SURVIVAL IN PATIENTS WITH PANCREATIC CANCER

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BACKGROUND-AIM

Circulating tumor-derived extracellular vesicles (EVs) represent a dynamic source of tumor-associated biomarkers. Accordingly, analysis of phenotypes and cargo of circulating extracellular vesicles may provide valuable tools for cancer diagnosis, prognostication, and surveillance. In our study, we explore the clinical relevance of blood circulating extracellular vesicles with a tumor-like phenotype (CD45-EPCAM+) in patients with pancreatic cancer (PC). Furthermore, we evaluated the correlation between surface PD-L1 expression on circulating CD45-EPCAM+ EVs and clinical and pathological factors in the enrolled cohort.

METHODS

This multicentric prospective study recruited patients with histologically or cytologically confirmed diagnosis of pancreatic cancer. Patients were recruited from June 2020 to May 2022. EVs were identified, enumerated, and phenotypically characterized from fresh whole blood samples by applying a polychromatic flow cytometry (FC) protocol based on a combined EV staining with a lipophilic cationic dye (LCD) and phalloidin. Flow cytometry surface subtyping of total EV population was performed by combined selection of EPCAM positivity and CD45 negativity. CD45-EPCAM+ events were further characterized based on PD-L1 expression.

RESULTS

A total of 56 patients with PC (resectable [n=9]; borderline resectable/unresectable [47]) and 48 healthy controls (HCs) were enrolled. Median blood concentrations of CD45-EPCAM+PD-L1- EVs (median EVs/ul=21.0; 95% CI 16.2-28.3) were doubled in the cancer population compared to HCs (median EVs/µl= 15.0; 95 % CI 9.5-20.5) (p. 0.02). Furthermore, blood levels of CD45-EPCAM+PD-L1- EVs were almost 2-fold higher in patients with metastatic disease compared to localized PC (p 0.01). Univariate and multivariate survival analysis revealed a significant correlation between high CD45-EPCAM +PD-L1- EV concentration (>22.9 EVs/µl) and worse overall survival (HR= 6.1 [95 % CI 1.86-20.02]; p. 0.001) in patients with advanced PC.

CONCLUSIONS

Overall, our results provide exciting perspectives into the identification of blood-based EV biomarkers for improved clinical management of patients with pancreatic cancer. Furthermore, findings form this study support a promising application of PD-L1 evaluation for further enrichment of tumor-derived EVs in biological samples.

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P113

A GENE SIGNATURE DERIVED FROM GERMINAL CENTER DARK ZONE ASSOCIATES WITH T CELL DEPLETION IN CANCER

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BACKGROUND-AIM

The germinal centers (GCs) are characterized by a neat compartmentalization of resident cells in a dark zone (DZ) with highly proliferating Ki-67+ B lymphocytes, a light zone (LZ) rich in follicular dendritic cells and a small fraction of CD4+ and CD8+ T cells. The proliferative index and expression of activation-induced deaminase (AID) mark the DZ as a microenvironment prone to DNA damaging events related to replication stress and mutagenesis. We studied the microenvironmental composition of the DZ as a model of tissue niche rich in mutational events to identify determinants of the control of T infiltration and activation to be extended to the study of hematological and solid tumors.

METHODS

To identify the genes most expressed in DZ, we analyzed the in situ transcriptional profiling of paired DZ and LZ GC regions of interest exploiting Digital Spatial Profiling on human tonsil, probing 1824 curated genes allowing comprehensive profiling of immune responses and microenvironment.

RESULTS

We derived a spatial DZ/LZ signature consisting of 370 differentially expressed genes (169 UP-regulated in the DZ and 201 UP-regulated in the LZ). The DZ regions were characterized by the overexpression of DNA damage sensing, response and repair genes, allowing for efficient checkpoint control over immune cells activation, or immune exclusion. The DZ transcriptional profiling showed down-regulation of molecular pathways involved in immune activation and inflammatory cytokine signaling, and spatial analysis of CD3+ and AID+ cell distribution confirmed T-cell depletion in the DZ. The application of the DZ spatial signature to case cohorts of Diffuse Large B-cell Lymphoma (DLBCL) revealed a negative association between DZ transcriptional imprint and T-cell content. A core set of DZ genes were also found to negatively associate with T-cell signatures in solid cancer histotypes from The Cancer Genome Atlas (TCGA).

CONCLUSIONS

Our study offers new insight into the DZ/LZ immune interface. The results in DLBCL and TCGA solid cancer series suggest that molecular programs involved in the maintenance of genomic stability and chromatin organization of the DZ may cast a negative influence over T-cell infiltration in tumors, representing potential targets for “heating” a “cold” microenvironment, enabling immunotherapy.

P114

THE DUAL ROLE OF GLUTATHIONE TRANSFERASE OMEGA 1-1 (GSTO1-1) IN THE SENSITIVITY VS. RESISTANCE OF CANCER CELLS TO ARSENIC TRIOXIDE

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BACKGROUND-AIM

GSTO1-1 might modulate the efficacy of the anticancer drug As2O3 (ATO) in two opposite ways. Metabolism of ATO through GSTO1-1 in fact originates the more toxic metabolite methylarsonous acid. Nevertheless, in other instances (e.g., cisplatin) GSTO1-1 overexpression was associated with drug resistance. We have investigated such possible role(s) of GSTO1-1 in response to ATO treatments in in vitro experiments.

METHODS

HeLa GSTO1-1 stable transfected (HeLaGSTO1^{+}), HeLa control (HeLaCont) and HeLa CRISPR/Cas 9 ko (HeLaGSTO1^{-}) cells were used to test the toxicity (WST-1) of increasing doses of ATO. ROS production (DCFDA), apoptosis (Hoechst), autophagy (LC3, IB), electron microscopy (TEM) and GSTO1-1 localization (IHF) were also evaluated.

RESULTS

The survival of HeLaGSTO1^{+} cells was constantly higher than that of HeLaCont and HeLaGSTO1^{-} cells at all concentrations tested, suggesting that GSTO1-1 expression can efficiently protect cells against ATO toxicity. ATO was however less toxic in HeLaGSTO1^{-} than in HeLaCont cells, confirming that at lower levels of expression the GSTO1-1 mediated ATO metabolism can rather effect a bioactivation of the drug.

CONCLUSIONS

GSTO1-1 was shown in our laboratory to be involved in both cancer progression and cisplatin resistance [1, 2]. The present results with ATO suggest that at high GSTO1-1 expression, the balance between protection and ATO bioactivation is likely in favor of protection, while at basal GSTO1-1 expression ATO toxicity prevail. Neither apoptosis nor autophagy were detected, but rather a marked cytosolic vacuolization (TEM). IHF images showed an early nuclear translocation of GSTO1-1. Since GSTO1-1 plays a major role in protein glutathionylation [3], such observation might suggest a possible role in the modulation of DNA repair systems. Overall, it can be concluded thus that high levels of GSTO1-1 expression are a factor of resistance against ATO therapies.

1. Piaggi S, et al. Oncol Rep. 2009; 21: 283
2. Piaggi S, et al. Carcinogenesis. 2010; 31: 804
3. Board PG, et al. Arch Toxicol. 2016; 90: 1049

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THE CRL3REN/KCTD11/SALL4/HDAC1 AXIS PROMOTES HEDGEHOG-DEPENDENT MEDULLOBLASTOMA THROUGH GLI1 DEACETYLATION

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common paediatric brain tumor that arises from alterations in cerebellum development. MB shows a high molecular heterogeneity and is associated with poor prognosis. Multi-omics approaches distinguished four main MB molecular subgroups. The Sonic Hedgehog variant (SHH-MB) is the best genetically understood, characterized by mutations in key components of SHH signalling and cytogenetic alterations. We previously identified the Cullin3 BTB-containing adaptor REN^{KCTD11} (REN) as a tumor suppressor that maps on chromosome 17p, a genomic region lost in ~30% of human SHH-MBs. REN is involved in neural progenitor development and acts as key negative regulator of the SHH pathway by promoting ubiquitylation and degradation of HDAC1, a well known SHH pathway activator.

METHODS

Affinity purification coupled to mass spectrometry was performed to identify new REN interactors. Co-immunoprecipitation and ubiquitylation assays were conducted to validate proteomic data. The in vitro proliferation of primary SHH-MB cells from mouse models and Patient Derived Xenografts (PDXs) was evaluated with the IncuCyte® Live Cell technology. Hetero- and orthotopic allografts of SHH-MB were established in vivo in immunodeficient NGS mice.

RESULTS

We identified Spalt-like transcriptional factor 4 (SALL4) as a new REN interactor. SALL4 is mainly expressed in embryonic stem cells and implicated in the maintenance of pluripotency. In several human malignancies, SALL4 expression is reactivated in adult tissues and is often associated with worse prognosis and lower survival rate. Here, we found that $CRL3^{REN}$ induces SALL4 poly-ubiquitylation and proteasome-mediated degradation. Interestingly, SALL4 binds GLI1 (the final effector of the SHH pathway) and works in complex with HDAC1 to promote GLI1 deacetylation thus inducing its transcriptional activity. Of note, genetic depletion of SALL4 inhibits SHH-dependent tumor growth both in vitro and in vivo. Accordingly, high SALL4 expression levels correlate with worse overall survival in SHH-MB patients.

CONCLUSIONS

Our findings highlight the relevance of a key interplay between ubiquitylation and acetylation in the regulation of the SHH pathway and identify SALL4 as a novel $CRL3^{REN}$ substrate and a potential target for SHH-dependent cancer therapy.

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THE ACTIVATION OF JAGGED1 SIGNALING BY CHEMOTHERAPEUTIC AGENTS COUNTERACTS THE OXALIPLATIN/5FLUOROURACIL- MEDIATED ANTI-CANCER EFFECTS: A NOVEL MECHANISM OF DRUG RESISTANCE IN COLON CANCER.

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BACKGROUND-AIM

Colorectal cancer (CRC) is a leading cause of mortality worldwide, characterized by metastasis and resistance to therapy. Recently, we demonstrated that $Kras^{mut}$ drives the activation of Jag1-ICD oncogene, via-ERK1/2. Herein, we explore the new intrinsic drug-resistance mechanisms, Jag1-ICD-mediated.

METHODS

Human CRC cell lines were treated with different chemotherapeutic compounds (e.g. OXP, 5FU and GSIs), alone or in combination, and subjected to in-vitro assays, to evaluate proliferation, metastasis and chemoresistance. CRC resistant cells were obtained by chronical treatment with low doses of OXP/5FU. The resistant cells were analysed by colony-formation assays and by qRT-PCR to assess growth and gene-reprogramming ability.

RESULTS

Herein, we evaluate the effects of OXP, 5FU and GSIs alone or in combination, on Jagged1 processing in CRC cell lines. We demonstrate that the anticancer drugs, OXP and 5FU, lead directly to a massive Jag1-ICD activation that results in the selection of a drug-resistant subpopulation. The chemoresistance mechanism is induced by a forced Jag1-ICD accumulation that protects cells from apoptosis, under the activation of Jag1-ICD-dependent pro-survival targets. In addition, GSIs induce the proliferation of Jag1-ICD positive CRC cells, functioning as tumor-promoting agents. Finally, the Jagged1 abrogation in OXP- or 5FU-resistant subpopulations is enough to restore the sensitivity to chemotherapy, confirming that drug resistance is Jag1-ICD-dependent.

CONCLUSIONS

Overall, our data show that Jagged1 processing is directly activated by the most potent chemotherapeutic agents (OXP/5FU) or by GSIs compounds. Moreover, we unveil a new role for Jag1-ICD oncogene which controls both apoptosis and proliferation, in CRC cells upon chemotherapeutic treatments. Therefore, we demonstrate the existence of a new mechanism of intrinsic drug-resistance, where Jag1-ICD functions as pivotal nuclear effector. Finally, we suggest Jagged1 as molecular predictive biomarker for the chemotherapy-outcome in CRC patients bearing $Kras^{mut}$ and over-expressing Jagged1.

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PROTEOMIC ANALYSIS OF PANCREATIC CANCER-DERIVED EXOSOMES SUPPORTS THEIR POTENTIAL ROLE AS LYMPHOCYTE IMMUNE ACTIVATORS

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BACKGROUND-AIM

Pancreatic cancer (PC) is unresponsive to immunotherapy with checkpoint inhibitors and its microenvironment is highly immunosuppressive. Tumor-derived extracellular vesicles (EVs), including exosomes, play immunomodulatory effects and may activate or inhibit immune responses. Little is known about their immunomodulatory role in PC. Our study aimed at investigating the potential immunomodulatory effect of PC-derived exosomes on circulating lymphocytes.

METHODS

Capan-2 PC cells-derived exosomes (Exo) were isolated by ultracentrifugation and their sizes were analyzed by dynamic light scattering (DLS). Fresh PBMCs from healthy donors were treated with Exo and CD3+ lymphocytes were isolated by fluorescence-activated cell sorter. Exo and CD3+ lymphocytes Exo-treated or untreated were subjected to proteomic analysis.

RESULTS

Proteomic analysis of exosomes derived from Capan-2, identified 95 proteins that were connected in a single functional network (p=1x10-16) by STRING analysis. According to IPA, 83 of the 95 identified proteins were involved in extracellular exosome (FDR 4.80x10-64), confirming the exosomal origin of the protein dataset. Intriguingly, among exosomal proteins detected, mesothelin, HSP90 and HSP70 are involved in immune activation. The highest ranked IPA downstreams pathways were immune mediated inflammatory disease (p=1,84x10-21), leukocyte migration (p=3,74x10-16) and cell movement of lymphocytes (p=6,10x10-15), which are consistent with an activation of immune response.
Proteomic analysis of CD3+ lymphocytes treated with Exo identified 1065 proteins, 60 of which were not present in untreated lymphocytes. According to IPA Comparison Analysis of Exo-treated or untreated CD3+ lymphocytes, cell proliferation of T lymphocytes (z-score 3.48), cell movement (z-score 3.02) and immune response of cells (z-score 2.92) were the most activated downstream effects. In line with these downstream effects, IPA upstream regulator analysis highlighted interferon-# as one of the most activated upstream regulators in CD3+ lymphocytes treated with Exo.

CONCLUSIONS

Our proteomic analysis supports a potential role of exosomes as antitumor immunity enhancers in pancreatic cancer.
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LEISHMANIASIS IN HUMANS AND DOGS: MONOVALENT IONOPHORES AS POTENTIAL NEW DRUGS

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BACKGROUND-AIM

Leishmaniasis is a vector-borne parasitic disease affecting thousands of people, mainly in tropical poor countries. The disease is transmitted by a phlebotomine sand fly, and infected dogs represent the main reservoir hosts. Since no vaccine is available for humans, control is mainly based on chemotherapy. However, the increased drug resistance urgently requires the identification of new molecules. We have previously demonstrated that monovalent sodium and potassium ionophores, largely used in veterinary medicine and studied as human anticancer agents, have antimalarial activity against Plasmodium falciparum, the agent of malaria. In this work the activity of three monovalent ionophores, monensin, salynomicin and nigericin, was evaluated against promastigotes (the infective parasite stage) of different Leishmania spp and against L. infantum amastigotes (the intracellular form in mammalian hosts) in differentiated human or dog macrophages.

METHODS

The activity of ionophores against the promastigote stage and the cytotoxicity on different cell lines were assessed by MTT assay. Amastigotes were obtained by infecting with L. infantum promastigotes (1:10 cell:promastigote ratio) human THP-1 or dog mononuclear cells (obtained by dog whole blood by Ficoll separation), both differentiated to macrophages with PMA. Macrophage infection was measured by Giemsa staining and microscopic observation.

RESULTS

All the ionophores showed a dose-dependent activity against promastigotes of L. infantum, L. tropica and L. brasiliensis. Nigericin and monensin had comparable activity ($IC_{50} < 1\mu M$), higher than that of salinomycin ($IC_{50} \sim 5\ \mu M$). All the ionophores had an IC_{50} of about 1.5-2 μM on intracellular amastigotes differentiated within human cells, with a selectivity index higher than 30 for monensin and nigericin. When dog macrophages were infected with Leishmania spp., higher percentages of infection were obtained compared to human macrophages, but the effect of the ionophores was lower.

CONCLUSIONS

Although further studies are needed, the monovalent ionophores are promising anti protozoal agents for humans. The lower efficacy in dogs indicates the need for different and specific drugs in a one-health perspective.

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THE REPURPOSED DRUG NITROXOLINE INHIBITS TUMOR GROWTH AND SYNERGIZES WITH GEMCITABINE IN REDUCING LUNG METASTASES IN MOUSE MODELS OF PANCREATIC CANCER

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BACKGROUND-AIM

We previously identified nitroxoline as a promising repurposed drug candidate in pancreatic cancer (PC) treatment. In the present study, we first tested the impact of combining nitroxoline with the first-line treatment gemcitabine on PC cell viability. Then, we explored the effects of nitroxoline and gemcitabine, as single agents or in combination, in mouse models of PC.

METHODS

The effects of nitroxoline and gemcitabine, as single agents or in combination, on PC cell viability (AsPC-1, Capan-2, BxPC-3) were assessed by MTT. The antitumor potential of the two drugs, as single agents or in combination, was tested in immune-deficient NSG mice, xenografted subcutaneously with AsPC-1 cells. Mouse treatment groups were: 1) nitroxoline; 2) gemcitabine; 3) nitroxoline and gemcitabine combined; 4) vehicle. Treatments started when the tumors reached palpable volume of about 100 mm3. Mouse Grimace Scale score was assessed. At the end of the experiments, mice were euthanized, organs and tumors were fixed and embedded for histology and immunohistochemistry. Animal studies were approved by the Italian Ministry of Health.

RESULTS

Combinations of nitroxoline and gemcitabine affected PC cell viability in a dose-dependent manner and their effect exceeded those of single agents at the highest concentrations tested in AsPC-1 ($p < 0.0001$) and in Capan-2 ($p < 0.001$). In vivo, nitroxoline and gemcitabine treatments, as single agents, or in combination, had no adverse effects, as assessed by evaluation of body weight and analysis of organ toxicity. Notably, tumor xenograft growth inhibition was more marked with nitroxoline (49%) and gemcitabine (43%), as compared to their combination (24%). Remarkably, combinations of nitroxoline and gemcitabine synergistically decreased lung metastases (77%) more markedly than nitroxoline (50%) and gemcitabine (60%).

CONCLUSIONS

We show for the first time that nitroxoline inhibited pancreatic tumor growth in vivo, while the combination of nitroxoline with gemcitabine limited metastatic disease in a synergistic way. These results indicate that nitroxoline has the potential to expand therapeutic options for the currently unsatisfactory PC treatment.

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CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL IS ASSOCIATED WITH INCREASED HUMORAL IMMUNITY IN THE CALCIFIED AORTIC VALVE

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BACKGROUND-AIM

Clonal hematopoiesis of indeterminate potential (CHIP), is a frequent aging-related event, whereby hematopoietic stem cells (HSCs) acquire somatic mutations that drive clonal expansion in the absence of cytopenias and dysplastic hematopoiesis. CHIP has been associated with a pro-inflammatory state and linked to the acceleration of cardiovascular disease. Recently, CHIP was found to occur more frequently in patients with calcific aortic valve disease (CAVD), where it was associated with a poor prognosis after valve replacement.

METHODS

In this study, we assessed CHIP frequency in a cohort of 168 patients with calcified aortic stenosis undergoing surgical aortic valve replacement (SAVR) or transcatheter aortic valve implantation (TAVI) by targeted DNA resequencing in the blood cells, focusing mainly on 9 CHIP-driver genes. The presence and association of CHIP with survival 12 months after valve replacement was evaluated. To understand the molecular pathways linking CHIP to CAVD, transcriptome analysis was performed on surgically removed aortic valves from patients with or without CHIP and non-calcific controls.

RESULTS

We found that CHIP is a common event in CAVD patients, with a higher frequency in females than males, where survival analysis revealed that CHIP status is predictive of adverse outcomes in males, but not in females. A comparison of the valve transcriptomes from CHIP patients with non-calcific controls identified 266 differentially expressed genes. Most of these are involved in adaptive immune responses, with 67 genes related to B cell activation and immunoglobulins production. A direct comparison of CHIP and non-CHIP samples revealed alteration of rel IL-15 signaling, B cell development, and lupus in B cell signaling pathways.

CONCLUSIONS

This study indicates that CHIP is a common event in CAVD patients, and its presence is often accompanied by a broad cellular and humoral immune response in the aortic valve. The excessive inflammatory response in CHIP patients may be related to the onset and/or progression of CAVD and point to B cells as possible new effectors of CHIP-induced inflammation.

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METABOLOMICS AND MICROBIOTA ALTERATIONS IN INFLAMMATORY BOWEL DISEASES: FROM IN VIVO TO IN VITRO

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BACKGROUND-AIM

Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the intestinal tract, including ulcerative colitis (UC) and Crohn's disease (CD) whose pathological mechanisms are not fully known. IBD is characterized by changes in the intestinal mucosa and the enteric microbiota. Combined metabolomics and microbiota analyses of stool, biopsies, and plasma were conducted to investigate the differences between UC and CD. These combined evaluations may help to discriminate between healthy subjects and patients with IBD and better understand the underlying pathological mechanisms.

METHODS

Metabolomics in stool, biopsies, and plasma and the stool microbiota of 183 subjects (82 UC, 50 CD, and 51 controls) were studied. Plasma and biopsies were analyzed by GC-MS and LC-MS and stool samples by GC-MS and 1H-NMR. For microbiota analysis, 16S rRNA gene sequencing data were produced from each specimen by the Illumina Hi-Seq platform to reveal the gut microflora composition. Analysis of the data was carried out using the BaseSpace16S Metagenomics App (Illumina). Metabolomics data were analyzed by using multivariate statistical techniques. Nicotinic acid (NA) was tested on a Caco-2 in-vitro model in which inflammation was induced by IL-1β and LPS that stimulate the production of IL-8.

RESULTS

Significant differences were highlighted between IBD and controls in plasma, biopsies, and feces metabolome (e.g. energetic metabolism, amino acids, phosphatidylcholines, fatty acids, TMAO, and nicotinic acid). In the IBD group, Firmicutes, Proteobacteria, Verrucomicrobia, and Fusobacteria were significantly increased, whereas Bacteroidetes and Cyanobacteria were decreased. The discriminant metabolites showed a good correlation with the microbiota profile. In particular, among the metabolites altered in the feces, NA decreases in patients with IBD compared to controls. After treatment of Caco-2 cells with IL-1β and LPS, a protective effect by NA was observed by reducing the levels of IL-8.

CONCLUSIONS

Metabolomics may be considered a valid tool to improve our understanding of the physiopathological mechanisms causing IBD. The low levels of nicotinic acid and its anti-inflammatory effects suggest its supplementation in the treatment of IBD.

P122

TARGETING THE HEPARAN SULFATE BIOSYNTHESIS IN OVARIAN CANCER: THE ROLE OF EXT1.

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BACKGROUND-AIM

Exostosin Glycosyltransferase 1 (EXT1) is an enzyme involved in the extension of the saccharide chain of heparan sulfate proteoglycans (HSPGs), key components of the extracellular matrix (ECM) and cell surface. Remodeling of the ECM plays a critical role in cancer development and progression. The ECM profile is relatively unexplored in Epithelial Ovarian Cancer (EOC), and data on the involvement of the ECM in platinum resistance are lacking.

METHODS

Copy Number Alterations (CNAs), reflected by altered mRNA expression, were analyzed in EOC patients from the TCGA dataset and stratified in terms of response to platinum-based therapy into 14 refractory, 59 resistant, and 107 sensitive patients. EXT1 protein levels were assessed by immunohistochemistry on a microarray of 10 refractory, 15 resistant, and 25 sensitive patients. Ovarian cancer cells were transfected with an EXT1-expressing vector (COV318, MDAH2774) to study the deposition and distribution of HPSGs and to determine their effects on signal transduction, cell motility, invasion, and drug sensitivity.

RESULTS

EXT1 was more amplified in refractory patients compared to platinum-sensitive patients ($p < 0.01$). Immunohistochemical analysis revealed increased EXT1 levels in 60% of refractory patients compared to 24% of sensitive patients ($p < 0.05$). Our results indicate that EXT1 increases the synthesis of HS and influences the sensitivity of ovarian cancer cells to growth factors and platinum-containing drugs.

CONCLUSIONS

Our preliminary results suggest that altered HSPG biosynthesis may form a physical barrier that limits access of chemotherapeutic agents and contributes to the onset of drug resistance.
Given the existence of anticancer drugs that alter HPSG synthesis, targeting HPSGs is a promising strategy to treat resistant EOCs

P123

METABOLIC ALTERATIONS OF HCC ARE DEPENDENT UPON DIFFERENT ONCOGENE ACTIVATION

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BACKGROUND-AIM

Hepatocellular carcinoma (HCC) is a multi-step process whereby abnormally proliferating cancer cells often undergo metabolic reprogramming. Increasing evidences suggest that different metabolic alterations taking place in hepatocarcinogenesis are dependent upon the activation of specific oncogenes, partially explaining the HCC heterogeneity.

METHODS

Hence we aimed to investigate and characterize the metabolic reprogramming of tumors induced in C57BL/6J mice hydrodynamically co-transfected with c-Myc and h-Ras, two of the most frequently mutated oncogenes in human HCC. Then we compared the results with the metabolic alterations taking place in tumors induced by co-transfection of CTNNB1S45Y and k-RasG12V.

RESULTS

The two different oncogene combination led both to the formation of HCCs of variable size in 100% of mice. The results showed that c-Myc oncogene overexpression leads to a metabolic rewiring toward a Warburg phenotype. Tumors were indeed characterized by high glycolytic flux, as suggested by the up-regulation of Glut1, Hk2, Mct4 and by an increased activity of the Pentose Phosphate pathway (PPP), leading to the generation of large volumes of lactate, through lactate dehydrogenase A (Ldha), even in the presence of oxygen. In these tumors, glutamine synthesis was strongly hampered and accompanied by down-regulation of TCA cycle and OXPHOS, while its catabolism was enhanced in c-Myc-h-Ras tumors while its synthesis

A rather different metabolic pattern was observed in CTNNB1S45Y and k-RasG12V tumors. Indeed, these tumors, unlike c-Myc and h-Ras HCCs, preferentially used glutamine synthesis to cope their metabolic demand instead of the glycolytic pathway and PPP.

CONCLUSIONS

The results of the present work highlight that the metabolic alterations taking place in HCC are dependent upon different oncogene alterations. The specific metabolic alterations displayed by an individual tumor may contribute to better tumor stratification and improve decision making and the treatment choices.

P124

CHARACTERIZATION OF RESPONSE TO TYROSINE KINASE INHIBITORS (TKI) IN MEDULLARY THYROID CARCINOMA

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BACKGROUND-AIM

Medullary thyroid cancer (MTC) is a malignant thyroid tumor arising from thyroid C cells, with frequent mutations in the rearranged during transfection (RET) or RAS genes. The management of advanced and progressive MTC patients is still a challenge because therapeutic options achieve poor results. These patients were treated with tyrosine kinase inhibitors (TKI) that include Cabozantinib and the recent FDA approved selective RET inhibitor, Pralsetinib (Blu-667). A limitation to efficacy of TKI therapy is that tumor cells might develop an escape mechanism.
The aim of this study was to characterize the response to Blu-667 in 3 MTC cellular models that recapitulate tumor different aspects: the first cells in normoxia, the second cells in hypoxia (more aggressive) and the third the TKI resistant cells (those present in tumors under therapy).

METHODS

TT cells (MTC C634W RET mutated) were treated for 72h with 2,5 μM Cabozantinib or 50 nM Blu-667 in the presence or absence of hypoxia. Cell RET modifications, proliferation, apoptosis, PI3K/AKT, MAPK/ERK and other oncogenic signaling pathway as HH-Gli activation were assessed.

RESULTS

Blu-667 inhibited RET autophosphorylation and proliferation in both TT cells models in normoxia and hypoxia. In comparison with Cabozantinib, Blu-667 induced the activation of an apoptotic program and downregulated HIF-1a in hypoxic cells. Interestingly, Blu-667 inhibited RET downstream signalling pathways. Evaluating possible molecular escape mechanism, we decided to check if the signaling pathway of HH-Gli was activated in our models. Indeed, we observed by immunofluorescence, a subpopulation of hypoxic and normoxic TT cells where Blu-667 stimulated the re-localization of the active form of the transcription factor of the HH-Gli signaling, Gli1, into the cell nuclei. We obtained the same results in a third model of TT cells, the Blu-667 resistant cells established by treating them with increasing concentrations of Blu-667.

CONCLUSIONS

The new RET selective inhibitor, Blu-667, is more effective than the TKI inhibitor, Cabozantinib, in impairing MTC cell growth and inducing cell death in both normoxia and hypoxia models . Moreover, we demonstrated a new escape molecular mechanism to Blu-667 therapy.

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NEW INSIGHTS ON THE MOLECULAR MECHANISMS DRIVING GLIOBLASTOMA TUMORIGENESIS

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BACKGROUND-AIM

Glioblastoma (GB) is the most malignant primary brain tumor in human, with an overall survival of approximatively from 8 to 15 months. The extensive cellular and genetic heterogeneity, as well as its rapid progression, invasiveness and the occurrence of drug-resistant cancer stem cells, limits the efficacy of the current therapies making GB one of the most difficult tumor to treat. For these reasons, the identification and characterization of the molecular mechanisms involved in GB tumorigenesis represent a dramatic challenge for the development of more effective and innovative therapeutic approaches.

METHODS

A gene expression analysis on publicly available datasets has been performed to identify new molecular players responsible for the GB malignant phenotype. Co-immunoprecipitation, ubiquitylation and cycloheximide assays have been performed to define the mechanism of action of MEX3A in GB tumorigenesis. In vitro and in vivo proliferation assays have been carried out on GB human primary cells to examine the effect of its modulation on GB growth.

RESULTS

We found that Muscle Excess 3A (MEX3A), an RNA-binding protein and E3 ubiquitin ligase, is strongly up-regulated in GB specimens. High levels of MEX3A are associated with very low protein levels of the Retinoic acid-inducible gene I (RIG-I), a tumor suppressor involved in the activation of the innate immune system through the type I interferon (IFN) response, and in the induction of cell growth arrest via apoptosis. We demonstrated that MEX3A binds RIG-I and promotes its ubiquitylation and proteasome-dependent degradation. Further, the genetic depletion of MEX3A leads to an increase of RIG-I protein levels and results in the suppression of GB cell growth in vitro and in vivo. Interestingly, we observed that high levels of MEX3A correlates with worse overall survival and a poor prognosis. Consistently, we found a negative correlation between MEX3A and the mutated IDH1 status and a positive correlation between MEX3A and EGFR expression.

CONCLUSIONS

These evidences uncover MEX3A as a novel diagnostic and prognostic biomarker in GB and highlight a previously unknown role of MEX3A/RIG-I axis in GB tumorigenesis. In this scenario, targeting these proteins could open innovative perspectives for new multi-targeting approaches in the treatment of this devastating tumor.

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COX-2 IS SHUTTLED BY EXTRACELLULAR VESICLES RELEASED BY GLIOBLASTOMA CELLS: A SUGGESTED ROLE AS A MEDIATOR OF CHEMORESISTANCE AND MODIFIER OF THE TUMOR MICROENVIRONMENT

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BACKGROUND-AIM

The extracellular vesicles (EV) are essential actors in tumor microenvironment (TME), including glioblastoma (GBM), the most aggressive intracranial tumor. Even though remarkable advances in GBM therapy have driven significant progress, chemoresistance remains the main hurdle in patient survival. Temozolomide (TMZ), a DNA-alkylating and first-line drug for GBM treatment, slightly improves overall survival. Cyclooxygenase-2 (COX-2) is upregulated in TMZ-treated GBM cells promoting higher resistance. EV released by tumor cells, creating a communication network that modulates the TME, are chemoresistance mediators. Thus, in this study, we explored if EV from TMZ-treated GBM cells shuttled COX-2 to recipient cells modifying their phenotype. The effect of COX-2 inhibitors (COXIB), Celecoxib (CXB) and NS398, was also investigated.

METHODS

GBM cell lines, T98G and U87MG, were daily treated with TMZ for 5 days, to simulate the clinical condition. COXIBs were used alone or combined with TMZ under the same conditions. COXIBs' specificity was verified on GBM cell line U251MG, COX-2 null. Cell viability was assessed by trypan blue staining, COX-2 expression by western blotting and PGE2 levels by ELISA kit. The EV released by GBM cells were characterized by transmission electron microscopy, western blotting, and NanoSight (NTA) analysis. U937 macrophage and U251MG were used as recipient cells. Immunofluorescence staining verified the EV uptake, and the COX-2 shuttled in recipient cells. TGF-ß1 levels were quantified in the U937 supernatants by ELISA kit.

RESULTS

The COX-2 protein was present and actively transferred in GBM cell-derived EV. EV secreted by T98G cells, previously treated with TMZ, shuttled higher COX-2 levels and significantly increased the TGF-ß1 level, a pro-tumor M2-like phenotype in U937. COXIBs counteracted this effect. In addition, T98G-derived EV-COX-2 strongly affected the recipient TMZ-sensitive U251MG, making them significantly less responsive to TMZ cytotoxic action.

CONCLUSIONS

Our findings, confirming the crucial role of COX-2 in TMZ-resistance, provide the first evidence of the presence and effective functional transfer of COX-2 through tumor-derived EV, with consequences in TME modulation and chemoresistance transfer.

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NOVEL THYROID HORMONE RECEPTOR-BETA AGONIST TG68 SAFELY INDUCES HEPATIC FAT REDUCTION IN A NON-ALCOHOLIC STEATOHEPATITIS RAT MODEL

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BACKGROUND-AIM

Non-alcoholic fatty liver disease (NAFLD) encompasses a broad spectrum of hepatic pathological conditions ranging from simple steatosis to the non-alcoholic steatohepatitis (NASH), in absence of alcohol abuse or other causes of chronic liver disease. Patients affected by NASH can develop fibrosis, cirrhosis and, eventually, hepatocellular carcinoma (HCC). Despite the rising interest in finding a therapeutic strategy for this condition, no pharmacological approaches have been approved for the treatment of NAFLD yet. In this respect, the regulation of the thyroid hormone/ thyroid hormone receptor beta (THRß) pathway, offers a new potential therapeutic strategy as it participates in the hepatic lipid metabolism. Alteration of the hepatic THR signaling takes part of the rise and development of liver-associated diseases, such as NAFLD and HCC. To date, the use of THs as therapeutic approach is hindered by the lack of selectivity and adverse extrahepatic side effects, mainly due to their affinity for extrahepatic THRα receptors. The aim of this study is to test the effect of TG68, a newly-synthesized THRß agonist, on liver and extrahepatic organs.

METHODS

To selectively activate THRß, we synthesized a novel THRß agonist, namely TG68. Rats were fed a high fat diet (HFD) ad libitum for 28 weeks, and during the last three weeks animals received TG68 (2.8 mg/kg) dissolved in water. Blood and tissues, including liver, heart and kidney, were collected and analysed after sacrifice.

RESULTS

Three week-treatment with TG68 significantly reduced the liver weight/body weight ratio due to an increased fatty acid ß-oxidation and hydrolysis rates highlighted by the overexpression of Cpt1a and Pnpla2, respectively. Blood analysis showed reduced circulating glucose, triglycerides, and cholesterol. Moreover, TG68 reduced hepatic fat accumulation and ameliorates liver injury in the absence of extrahepatic toxic effects.

CONCLUSIONS

Rats fed a HFD and treated with TG68 for the last three weeks before sacrifice, showed a reduction in liver steatosis associated with an amelioration of the triglycerides and glucose levels. The antisteatogenic effect was associated with the activation of THRß and the induction of fatty acid ß-oxidation. The effect of TG68 on liver steatosis was confirmed by a robust reduction of intrahepatic lipid content. This study strongly suggests that TG68 is a novel hepatospecific THRß agonist, which might be used as a future therapeutic tool for the treatment of NAFLD.

P128

KCTD1: A NEW MODULATOR OF THE KCASH FAMILY OF ONCOSUPPRESSORS

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BACKGROUND-AIM

KCASH1, KCASH2 and KCASH3 oncosuppressors are involved in the negative regulation of Sonic Hedgehog (Hh) pathway, involved in embryonal development and in tumorigenesis. KCASH proteins can induce HDAC1 degradation, inhibiting transcriptional activity of Gli1, the main target and effector of the Hh pathway. Recently, we have discovered a new function of KCTD15 as a player in the complex network of regulatory proteins which modulate the Hh pathway by acting on the KCASH2 protein stability.
KCTD15 shares several features and biological functions with its paralogue KCTD1. This homology prompted us to verify if also KCTD1 may be involved in the regulation of KCASH and in the modulation of Hh pathway.

METHODS

KCTD1 interaction with KCASH1 and KCASH2 was verified by co-immunoprecipitation assays. WB analysis have been used to evaluate KCTD1 roles on KCASH1 and KCASH2 protein stability. Luciferase assays, RT-qPCR and WB analysis were performed to characterize the KCTD1 function in Hh pathway regulation. The KCTD1 function in nuclear-cytoplasmic transport of KCASH1 and KCASH2 was verified by nuclear/cytoplasmic extracts and WB analysis.

RESULTS

We demonstrate that KCTD1 is able to bind both KCASH1 and KCASH2 proteins, enhancing their stability and improving their inhibitory effect on Hh activity. Indeed, KCTD1 overexpression induces a decrease of HDAC1 protein levels, an increase of Gli1 acetylation, repressing Gli1 activity. Furthermore, we have observed that the subcellular localization of KCASH1 and KCASH2 is significantly shifted from nuclear to cytoplasmatic upon KCTD1 overexpression.

CONCLUSIONS

The ability of KCTD1 to act as a modulator of both KCASH1 and KCASH2, unlike the paralogue KCTD15, unveils a crucial role for KCTD1 in the negative regulation of the Hedgehog pathway. The characterization of the KCTD1 function in the regulation of KCASH stability will contribute to better understanding the mechanisms involved in the modulation of the Hh pathway, and to hypothesize the new therapeutic approaches in the treatment of Hh-dependent tumors.

P129

NOVEL RESVERATROL ANALOGUE EXERTS MARKED ANTIPROLIFERATIVE ACTIVITIES AND AFFECTS CD133+EPCAM+ CANCER STEM-LIKE CELL SUBPOPULATION IN PANCREATIC CANCER CELLS

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BACKGROUND-AIM

Resveratrol (RSV) is a natural polyphenol that exhibits multiple biological activities, including anticancer properties. Unfortunately, RSV displays a low oral bioavailability, limiting its in vivo effects. Here, we evaluated the physicochemical properties of novel RSV derivatives and explored their antiproliferative effects on pancreatic cancer (PC) cells.

METHODS

The effects of RSV derivatives on viability and self-renewal capacity of three PC cell lines (AsPC-1, BxPC-3 and Capan-2) were assessed by MTT and clonogenic assays, respectively. Polychromatic flow cytometry was applied to investigate the impact of compounds on PC stem-like cell subpopulations. The effects of compounds on PC cell cycle and apoptosis were assessed by flow cytometry and western blot (WB). Drug-like properties were explored by cLogP calculation, enzymatic stability assays and parallel artificial membrane permeability assay (PAMPA) for the evaluation of gastro intestinal (GI) absorption.

RESULTS

RSV derivatives inhibited PC cell viability with IC50 values lower than those obtained with RSV. Remarkably, DF5 derivative induced a significant and consistent reduction of the $CD133^+EpCAM^+$ cancer stem-like PC cell subpopulation, leading to drastic effects on cell clonogenicity. Moreover, DF5 strongly interfered with PC cell cycle progression and induced apoptosis and DNA damage, as assessed by WB analysis of cell cycle proteins, PARP cleavage and H2AX phosphorylation. Of note, DF5 exhibited no toxicity on HFF-1. Regarding drug-like properties, DF5 has both higher stability in human plasma and lipophilicity, as compared to RSV, which might ensure a better permeation along GI tract.

CONCLUSIONS

The RSV derivative DF5 exhibits prominent antiproliferative effects in PC cell lines, by affecting key processes in cancer cell biology. In particular, this compound strongly affects the subpopulation of PC cells with a $CD133^+EpCAM^+$ stem-like phenotype, indicating its potential in inhibiting self-renewal capacity of PC cells. Overall, our results provide insights into mechanisms of action contributing to the antiproliferative activity of DF5 and support the value of the compound in the search for effective and safe agents for PC treatment.
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P130

CHARACTERIZATION OF THE HECT-E3 UBIQUITIN LIGASE SMURF1 AS A NEW POSSIBLE MODULATOR OF THE HEDGEHOG SIGNALING PATHWAY

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BACKGROUND-AIM

GLI1 is a transcriptional factor known as the main effector of the Hedgehog (Hh) signaling pathway, which is involved in cell proliferation and differentiation along embryonic development. During the physiological activation of the Hh signaling pathway, as well as in pathological contexts, GLI1 is able to activate the transcription of genes involved in cell proliferation. Deregulation of the Hh pathway has been widely associated with tumorigenesis and understanding mechanisms regulating the Hh pathway has become crucial in trying to find new therapeutic approaches, against its pathological deregulation. We have previously identified the HECT-E3 Ubiquitin Ligase SMURF2 as a new negative modulator of GLI1 and given the functional similarity that distinguishes the different proteins of the SMURFs family, we recently investigated the potential role of SMURF1 in this context

METHODS

The effects of SMURF1 overexpression on GLI1 and Hh pathway have been investigated in HEK293T cells and in the human colorectal carcinoma cell line HCT116. Cells transfection has been made using liposomal based reagents. Protein levels have been analyzed by Western blot (WB). Hh pathway modulation has been monitored by RT-qPCR and luciferase reporter assays. GLI1 ubiquitination levels have been monitored by transfection of HA-tagged Ubiquitin, followed by immunoprecipitation assay (IP). GLI1-SMURF1 interaction was observed by Co-IP

RESULTS

We observed that overexpression of SMURF1 in vitro, led to a reduction of GLI1 protein levels. We demonstrated a negative effect of SMURF1 overexpression on Hh transcriptional activity by RT-qPCR and luciferase assay. Co-IP assay has demonstrated an interaction between SMURF1 and GLI1 protein. We proved, through ubiquitination assay, that SMURF1 is able to reduce GLI1 protein levels modifying its ubiquitination status. Our results suggest that SMURF1 overexpression increases GLI1 ubiquitination levels, and this modification leads to GLI1 degradation

CONCLUSIONS

Our results suggest a previously unknown inhibitory role of SMURF1 on GLI1 and on the Hh signaling pathway. Our evidence shows SMURF1 as a new negative modulator in the network of regulatory proteins which modulate GLI1 and the Hh pathway. Our observations demonstrate that SMURF1 and SMURF2 may act in similar fashion to modulate GLI1 and the Hh pathway. This redundancy may account for a tight and finely balanced control of Hh pathway, and it needs to be taken into account in the development of new therapeutic approaches

P131

THE EFFECTS OF DENTAL PULP STEM CELLS ON SCAR FIBROBLASTS IN A CO-CULTURE MODEL

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BACKGROUND-AIM

Fibrosis is characterized by delayed deposition and remodeling of the extracellular matrix (ECM). This condition is prompted by a chronic stimulus mediated by pro-inflammatory cytokines, such as TNF-α and IL-1β (released by macrophages) that synergistically initiate the inflammatory cascade inducing the fibroblasts (FBs) activation. Mesenchymal stem cells (MSCs) present an immunosuppressive and anti-inflammatory potential. MSC could be considered a promising therapeutic strategy for many chronic inflammatory diseases. However, the immunomodulatory role of MSCs and that of dental pulp stem cells (DPSCs) in fibrosis is still largely unknown. We aimed to clarify if DPSCs can affect FBs collagen deposition by modulating the inflammation process.

METHODS

FBs and DPSCs were co-cultured by using a transwell system and were stimulated with a TNF-α/IL-1β or LPS, as control. The relative expression of genes involved in the inflammasome activation or the deposition of fibrotic scar tissue (IL-1β, IL-6, IL-18, NLRP3, NAIP, AIM2, CASP1, PYCARD, TLR4, COL1A1, COL3A1, FN1, and TGFβ1) was quantified by RT-PCR after 4 h. The expression of IL-6, FN1 and MMP9 protein was assessed by Western-Blot, after 24 h.

RESULTS

Co-cultured FBs stimulated with cytokines showed a significant increase of IL-1β, IL-6, NAIP, and AIM2, compared to unstimulated cells. CASP1 was increased in co-cultured FBs treated with TNF-α, IL-1β, and LPS, indicating a possible role of DPSCs in modulating genes involved in the inflammation. We found a lower relative expression of IL-18 in co-culture FBs than in FBs alone. After stimulation, DPSCs seem to induce FBs mRNA expression of FN1 and TGFβ. Conversely, western blot analysis, showed a decrease in FN1 production after 24h, suggesting potential post-transcriptional mechanisms of regulation induced by DPSCs. Cytokines-stimulated co-cultured FBs increased the production of matrix-metalloproteinases (MMP)-9, suggesting a role of DPSCs in ECM remodeling.

CONCLUSIONS

Our data suggest an in vivo crosstalk between FBs and DPSCs, which leads to the modulation of genes involved in ECM deposition, inflammasome activation, and, thus, a potential role in wound healing and fibrosis regulation. In conclusion, our results could pave the way for further studies to go deeper into the time-dependent interactions between FBs and DPSCs and the possible role of DPSC in modulating fibrosis.

P132

ROLE OF ASTROCYTE SENESCENCE IN THE GENERATION OF SEX DIFFERENCES IN ALZHEIMER'S DISEASE

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BACKGROUND-AIM

Alzheimer's disease (AD) is a neurodegenerative disorder with greater prevalence and incidence in women, who show faster cognitive decline than men and this discrepancy increases with advanced age. A crucial molecular mechanism involved in aging and neurodegenerative disease is cellular senescence, characterised by a pro-inflammatory secretome that could play an essential role in Alzheimer's pathogenesis by promoting neuroinflammation. We have developed a model of cellular senescence induced by $A\beta_{1-42}$ oligomers on primary astrocytes from AD patients and healthy patients of both sexes, trying to mimic in vivo conditions.

METHODS

Human primary astrocytes from healthy subjects and AD patients of different ages and sex (provided by Celprogen, USA) were treated with $A\beta_{1-42}$ oligomers (10μM) and cultured in a medium supplemented with 2% FBS for 5 days. The senescent phenotype was evaluated by analysing the expression of senescence-associated β-galactosidase activity (SA-β-gal), $p14^{ARF}$ expression, a gene involved in cell cycle arrest and the presence of pH2AX nuclear foci, a marker of DNA double-strand breaks frequently observed in senescent cells.

RESULTS

1) Astrocytes from AD patients after $A\beta_{1-42}$-treatment show a significant increase in SA-β-gal activity and p14 expression compared to healthy subjects. 2) Astrocytes from AD females reveal a significant augment of the markers mentioned above compared to AD males. 3) No differences in senescence propensity are evidenced in male and female healthy subjects. 4) $A\beta_{1-42}$-treated astrocytes from AD people of both sexes show a significant increase of pH2AX compared to healthy subjects. 5) No correlation between SA-β-gal activity and age was observed in AD patients and healthy subjects.

CONCLUSIONS

In this study, we have observed that astrocytes from AD patients, particularly females, show an increase in $A\beta_{1-42}$ induced senescence compared to healthy subjects. The increased astrocyte senescence in AD females could lead to a more detrimental milieu, able to induce neuron death. Our results could help explain the increased risk of developing AD in females, and investigating the molecular mechanisms involved in male and female astrocyte senescence might help us better understand neurodegeneration pathogenesis.

P133

SOLUBLE P2X7R AND NLRP3: POSSIBLE ROLE IN PATHOGENESIS AND SEVERITY OF COVID-19

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BACKGROUND-AIM

The Corona Virus Disease 2019 (COVID-19), is caused by infection from the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). A robust inflammatory response induced by viral replication is suggested as the main cause for the acute lung and multiorgan injury, particularly dramatic in several critical patients. Among different factors involved in immune and inflammatory responses, inflammasomes are suggested as main players in COVID-19 pathogenesis.

METHODS

We examined sera samples from 97 patients with confirmed SARS-CoV-2 infection presenting various degrees of COVID-19 disease severity. Sera were obtained from coagulated blood by centrifugation at 4°C for 15 min at 1000xg and concentrations of Interleukin 1-β (IL-1β), Interleukin 6 (IL-6), Interleukin 10 (IL-10), soluble P2X7R (sP2X7R) and soluble NLRP3 (sNLRP3) were determined using ELISA assays. Additionally, routine blood parameters were measured on the same samples at the Laboratory of Chemical analysis of the S. Anna Hospital of Ferrara using standard analysis methods.

RESULTS

The main finding of this study is that sP2X7R and sNLRP3 were increased in plasma of 61 and 74% of patients, respectively. Study of correlations showed that sP2X7R negatively and significantly correlated with age (p=0.017), lymphocytes (p=0.046), and eosinophils (p=0.018). sNLRP3 significantly correlated with leukocytes (p=0.038), neutrophils (p=0.032), and monocytes (p=0.004). Moreover, sP2X7R showed correlation with sNLRP3 at the limit of significance (p=0.057), and significantly correlated with IL-10 (p=0.008), PCT (p=0.009) and BNP (p=0.007). sNLRP3 also showed significant correlation with different inflammatory parameters, that is IL-6 (p=0.001), IL-10 (p=0.035) and CRP (p=0.028).Finally, sP2X7R was significantly increased in patients who presented fever and respiratory symptoms at debut (p=0.024), underwent hospitalization in Pneumonology division (p=0.031), required mechanical ventilation (p=0.005) or died during hospitalization (p=0.015). sNLRP3 was significantly higher in patients who required mechanical ventilation (p=0.039), and in those who underwent hospitalization in Intensive care unit (p=0.034).

CONCLUSIONS

sP2X7R and sNLRP3 are increased in sera of COVID-19 patients and correlate with other inflammatory and clinical parameters relevant in the pathogenesis of the disease. This suggests that sP2X7R and sNLRP3 might have a role in disease progression and their determination might have a significance as marker of disease severity.

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MICRORNA EXPRESSION ANALYSIS, TARGET GENES AND PATHWAYS INDUCED BY RHNGF IN CORNEAL EPITHELIAL CELLS.

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BACKGROUND-AIM

Nerve Growth Factor (NGF) plays a key role in the maintenance and functions of both the central and peripheral nervous systems, as well as in non-neuronal cells. NGF efficacy was demonstrated for corneal lesions treatment and recombinant human NGF (rhNGF) was approved for neurotrophic keratitis therapy. MicroRNAs (miRNAs) are involved in maintaining cell homeostasis and in several pathological processes. Here, we evaluated miRNAs' expression modulation in human corneal epithelial cells after time-dependent rhNGF treatment, in order to shed light on some of the molecular mechanisms, still unknown, at the base of NGF biological activity.

METHODS

Nearly 700 microRNAs were analysed by qRT-PCR. MiRNAs showing significant expression differences were examined by DIANA-miRpath v.3.0 to identify target genes and pathways. Immunoblots were performed to preliminarily assess the strength of the in silico results.

RESULTS

Twenty-one microRNAs were significantly regulated in response to rhNGF, most of which were never described in corneal cells. In silico analysis evidenced interesting target genes and pathways, including that of neurotrophin, which was further analyzed in depth. Almost 80 unique target genes were identified (e.g., PI3K, AKT, MAPK, KRAS, BRAF, RhoA, Cdc42, Rac1, Bax, Bcl2, FasL), most of them targeted by more than one miRNA, and playing a role in relevant signalling processes (i.e., MAPK, PI3K/AKT, NF-kB) and cell functions (e.g., differentiation, survival, or apoptosis) already described in corneal physiological and pathophysiological processes at the base of injury, wound healing, and regeneration. AKT and RhoA immunoblots demonstrated congruence with related microRNA expression levels, providing preliminary validation of in silico data.

CONCLUSIONS

MicroRNA levels in response to rhNGF were for the first time analyzed in corneal epithelial cells, by focusing the attention on target genes and pathways. Overall, the study provided novel insights about molecules and possible responses induced by this neurotrophin in cornea. Given the putative role of miRNAs as biomarkers or therapeutic targets, this study makes available data potentially exploitable in clinical practice.

P135

ANTI-FIBROBLAST AUTOANTIBODIES IN IGG4-RELATED DISEASE PATIENTS

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BACKGROUND-AIM

IgG4-related disease (IgG4-RD) is a rare fibro-inflammatory condition that can affect almost any organ, characterised by tissue fibrosis with a storiform pattern, a diffuse lymphoplasmocytoid infiltrate, obliterative phlebitis and abundance of IgG4-positive plasma cells.
Increased IgG4 levels and oligoclonal IgG4-producing plasmablasts in peripheral blood suggest the presence of a strongly skewed antigen-driven B cell response. Despite suggestive data for the role of B cells and serum antibodies in IgG4-RD, the antigenic specificity of IgG4+ plasmablasts has not been thoroughly analyzed or connected with disease-inducing mechanisms.
So far, limited information is available on the specificity of autoantibodies in IgG4-RD sera.
The aim of the present project is to analyse the role of autoantibodies in affecting the fibroblast phenotype and functions.

METHODS

Total IgG and IgG4 were obtained from IgG4-RD patients by means of Protein G affinity chromatography. Total IgG obtained from healthy subjects were used as controls.
Recombinant monoclonal antibodies (rmAb) were generated from IgG4-RD patients B cells, by single cell PCR and expression vector cloning.
Primary human dermal fibroblasts were isolated from the normal skin of subjects undergoing reductive bilateral mastectomy.
The role of patients IgGs in fibroblast cytokine production and fibroblast migration was evaluated by IgG-HDF coculture and scratch wounding assay

RESULTS

Anti-fibroblast antibodies are present in 9/23 (40%) of IgG4-RD as compared with 1/8 (12.5%) of healthy controls. Some of the rmAbs obtained from patients strongly reacted with dermal fibroblasts by immunofluorescence.
IgG4-RD IgG induces in dermal fibroblasts the production of cytokines, in particular IL-8, as compared with control IgGs.
Western blotting analysis confirmed the presence of anti-fibroblast antibodies in IgG4-RD patients.
Two of the rmAbs obtained from IgG4-RD patients promoted cell migration as compared with control IgGs

CONCLUSIONS

These results suggest that IgG4-RD patients develop anti fibroblast antibodies endowed with autoimmune potential and able to modify fibroblast behaviour, potentially driving the fibrotic process in IgG4-RD patients.

P136

GLUCOSE-DEPENDENT EFFECTS OF IINSULIN RECEPTOR ISOFORMS A AND B ON TAMOXIFEN RESISTANCE IN BREAST CANCER CELLS

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BACKGROUND-AIM

Breast cancer (BC) is the most common malignancy in women, and is linked to several risk factors including obesity, estrogen signaling, insulin levels and glucose metabolism. Insulin and Insulin-like growth factor (IGF) signaling are classified as mitogenic and pro-survival pathways. Pre-clinical studies have shown their involvement in the development, progression and resistance to treatment of several tumors, including BC. Insulin/IGF signaling is triggered by two insulin receptors (IR) isoforms identified as IR-A and IR-B and by Insulin-like growth factor receptor I (IGF-1R), which can initiate the intracellular signaling cascade alone or forming hybrid (IGF-1R/IR-A, IGF-1R/IR-B, IR-A/IR-B) complexes. While the role of IGF-1R in BC progression is well established, the effects of the two IR isoforms in this biological context have not been fully understood. Using estrogen-dependent MCF7IGF-1RKO BC cell models, selectively expressing IR-A (MCF7IGF-1RKO/IRA) or IR-B (MCF7IGF-1RKO/IRB), we investigated the effect of insulin stimulation on the anti-proliferative activity of tamoxifen (TAM) in presence of low (LG) and high (HG) glucose concentrations.

METHODS

MCF7$^{IGF\text{-}1RKO}$ cells were generated employing the CRISPR System. MCF7I$^{GF1\text{-}RKO/IRA}$ and MCF7$^{IGF\text{-}1RKO/IRB}$ were obtained from MCF7$^{IGF\text{-}1RKO}$ lentivirally transduced to selectively express IR-A or IR-B. Insulin IC50 values and proliferation rates were measured using the MTT assay. Apoptosis and cell cycle analysis were monitored by flow-cytometry. Clonogenic potential was investigated employing colony-forming unit assays and gene expression profiling by Q-PCR using a PCR array system.

RESULTS

We observed that HG increased the IC50 value of TAM for both IR-A and IR-B-expressing BC cells and that IR-B displayed a stronger anti-apoptotic effect than IR-A. However, IR-A exhibited a higher potency than IR-B in all other biological assays. Indeed, regardless of the glucose concentrations, IR-A was the most effective insulin receptor isoform in restoring cell-cycle progression blocked by TAM after insulin stimulation. Likewise, MCF7I$^{GF\text{-}1RKO/IRA}$ were more clonogenic than MCF7$^{IGF\text{-}1RKO/IRB}$ with both HG and LG. Finally, expression profiling arrays showed that insulin stimulation positively modulates genes involved in apoptotic signaling after TAM exposure.

CONCLUSIONS

Our data indicate that glucose levels strongly influence insulin receptor activity in BC cells, modulating TAM-mediated therapeutic effects.

P137

CIRCULATING MICRORNAS PROFILING IN FAMILIAL-HEREDITARY BREAST CANCER PATIENTS.

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BACKGROUND-AIM

Breast cancer (BC) is the most common cancer in women worldwide and the leading cause of cancer-related death. Approximately 5–10% of BC cases are classified as familial or hereditary, 25% of which are associated with germline mutations in the high penetrance susceptibility genes BRCA1 and BRCA2. Here, we performed a circulating microRNA (miRNA) profiling in a series of familial-hereditary BC cases in order to identify differentially expressed miRNAs with putative value of non-invasive biomarkers and to deepen the molecular mechanisms involved in the pathogenesis of this specific subgroup of BCs.

METHODS

Plasma miRNAs of 21 familial BC cases, comprising 14 BRCA1/2 positive (BRCA) and 7 BRCA1/2 negative (non-BRCA) BCs, were analyzed by miRNA-sequencing using Illumina technology. Three age-matched healthy controls were also included in the study.
A bioinformatic pipeline comprising Bowtie1 tool for alignment to miRBase v.22 and the reference genome GRCh38, as well as DESeq2 package for differential expression analysis was used. In silico analysis of the target genes/pathways of differentially expressed miRNAs was also performed.

RESULTS

miR-320e and miR-486-3p emerged as the most relevant miRNAs able to discriminate BC cases and healthy controls. Different expression levels of mir-486-3p were also observed related to BC molecular subtypes.
A panel of 20 differentially expressed miRNAs between BRCA and non-BRCA cases was identified, of particular relevance for the non-BRCA group.
In silico analysis of target genes/pathways of the differentially expressed miRNAs revealed an enrichment of genes involved in different cancer types (e.g. prostate, lung, melanoma) and key biological processes, including cell cycle, apoptosis, focal adhesion and growth factor signaling pathways.

CONCLUSIONS

Overall, these preliminary results suggest that the analysis of the expression levels of circulating miRNAs in familial-hereditary BCs, based on the BRCA1/2 germline mutational status, could provide, once extended and confirmed on a larger number of patients, important information of biological and clinical relevance, potentially exploitable in the context of screening and prevention programs.

P138

HYPOXIA-INDUCED LIPID METABOLISM SUPPORTS TUMOR ANGIOGENESIS IN GASTRIC CANCER.

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BACKGROUND-AIM

Gastric cancer (GC), currently the fourth leading cause of cancer-related death and the sixth cancer for incidence globally, is most dependent on vascularization for both nutrients uptake and escape from chemotherapy. GC continuous and uncontrolled growth is commonly paired by the onset of a dysregulated vascularization, resulting in a severely hypoxic microenvironment which in turn triggers cancer cells to undergo a deep metabolic switch, stimulating glycolysis and lactate secretion, inhibiting mitochondrial metabolism and, lastly, stimulating fatty acids (FA) synthesis as a survival mechanism. FA, complexed by cancer cells in the form of lipid droplets (LD), might be fundamental not only for the cell survival in a hostile environment but might also sustain the aberrant tumor vascularization.

METHODS

The AGS and 23132/87 cell lines were cultured under severely hypoxic conditions (0.1% O_2) for 24 hrs (acute hypoxia) or 96 hrs (chronic hypoxia). Tetramethylrhodamine (TMRM) was exploited via flow cytometric analysis to determine the relative mitochondrial membrane potential ($\Delta\psi$m), whilst ATP levels were measured by luminometry. The intracellular FA content has been measured via Nile Red staining and vascular morphogenesis experiments were conducted by treating human endothelial cells with GC cells-derived conditioned media enriched with LD. Western blotting and qPCR were used to evaluate markers associated with the hypoxia-induced FA-metabolism.

RESULTS

Acute hypoxia induced FA synthesis, via HIF-1α activation, while chronic hypoxia partially stimulated β-oxidation, thereby apparently providing energy and intermediates necessary for GC cell adaptation to low oxygen tension. The increased FA production conferred GC cells a metabolic advantage once oxygen was restored. The media collected from GC cell cultures incubated in low oxygen, with respect to the ones in normoxia, were proved to be enriched in LD, which in turn enhanced endothelial cell morphogenesis.

CONCLUSIONS

Our data demonstrated that GC cells are capable to survive at low oxygen tension, inducing FA synthesis which are then exploited lately when oxygen is provided back. Indeed, the produced LD are also capable to act as messengers in recruiting and stimulating endothelial cells.

P139

MAXIMIZING PLATINUM-BASED CHEMOTHERAPY IN OVARIAN CANCER BY INHIBITING THE PEPTIDYL-CIS/TRANS ISOMERASE PIN1

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BACKGROUND-AIM

Among gynecological malignancies, Ovarian Cancer (OC) is one of the most lethal. Cytoreductive surgery and Platinum based chemotherapy represent the standard treatment for OC-bearing patients. Nevertheless, chemotherapy is challenged by drug resistance. Notably, an increasing number of studies proved the role of Notch receptors in Platinum resistance. Among the four Notch receptors, it has been demonstrated that Notch3 (N3) is altered in a wide panel of OC, and it confers Platinum resistance to OC cell lines. Therefore, effective targeting of N3 may restore Platinum sensitivity. In this scenario, we previously demonstrated that peptidyl-cis/trans isomerase Pin1 positively regulates N3 in T-cell acute leukemia (T-ALL) and we wondered whether this relationship occurs also in OC context. Collectively, the main aims of the present work are to investigate in vitro and in vivo: 1. the N3-dependent Platinum resistance and 2. the potential role of the down-modulation of the Pin1-N3 axis to restore Platinum sensitivity.

METHODS

N3-positive and N3-negative established OC cell lines were used. We performed: 1. in silico analysis on mRNA data collected by OC-bearing patients; 2. immunohistochemistry (IHC) on OC tissue samples; 3. lentiviral transductions; 4. in vitro studies such as pharmacological treatments, cell viability and colony formation assays; and 5. in vivo experiments: xenografts in NOD/SCID immuno-deficient mice.

RESULTS

While in vitro and in vivo xenograft experiments proved the relevance of N3 in sustaining platinum drug resistance, protein expression and immunohistochemical analyses of primary tumors from OC-bearing patients showed that N3 over-expression significantly correlate with Pin1 high protein level, which was consistent with a worse prognosis. Furthermore, we demonstrated that the expression of N3 intracellular domain is sustained by Pin1 in OC cell lines These observations corroborate our hipothesis that the pharmacological inhibition of Pin1 may defect N3 signaling also in OC context.

CONCLUSIONS

All in all, clinical implications of our findings are relevant for OC-bearing patients since Pin1 targeting may aid in maximizing Platinum-based therapies efficacy.

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LOSS OF KCASH2 RESULTS IN AN INCREASED NEUROGENESIS RATE INSIDE THE DENTATE GYRUS OF HIPPOCAMPUS IN MOUSE MODEL

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BACKGROUND-AIM

The oncosuppresor protein KCASH2 has been described as a negative regulator of the Sonic Hedgehog pathway. Using a KCASH2 KO first mouse we have demonstrated that KCASH2 protein is expressed in the Brain, including the Hippocampus. In this region neurogenesis occurs troughout the life of mammals at the level of Dentate Gyrus (DG), where Neural Precursor Cells start to proliferate inside the hilus of DG and then migrate and differentiate in neurons forming the Granule Cell Layer. Deregulation of mechanisms underlying the maintenance of the homeostasis mechanism that regulate the proliferation rate of NPCs in the Hippocampus has been correlated with an impaired learning and memory formation. Characterization of the role of KCASH2 in the modulation of the Hh pathway and the staminality of neural cell in the dentate gyrus of hippocampus is therefore of great interest.

METHODS

In order to evaluate the expression of KCASH2 protein, we performed enzymatic β-galactosidase reporter assay in mouse tissue slides and western blot analysis. We compared the morphology and the proliferation rate of NPCs between WT and KCASH2 KO mice trough indirect Immunofluorescence (IF) using antibodies recognizing Glial acidic fibrillary protein (GFAP), Nestin and Ki-67. Western Blot analyses of tissue lysates have been performed to asses the expression levels of the components of Hh pathway.

RESULTS

Ex vivo Immuonfluorescence on mouse brain sections indicates an increased proliferation rate of Neural Precursor Cells inside the hilus of the Hippocampus of KCASH2 KO mice compared to WT. Moreover KCASH2 KO NPCs exhibits altered GFAP and Nestin filaments, suggesting an impaired abilty to differentiate. Western Blot analysis performed on dissected hippocampus have shown that the morphogen Shh is not modulated in presence or absence of KCASH2. We also present here the different expression levels of other components of the Hh pathway.

CONCLUSIONS

Our results suggest a role for KCASH2 in regulating the homeostasis and the proliferation of Neural Precursor Cell inside the hilus of the Dentate Gyrus. The proliferation rate of NPCs seems to be increased in KCASH2 KO mice in comparison to WT. The process trough wich KCASH2 modulate the neurogenic process involves the modulators of Hh pathway, but the mechanisms of this process need further characterization.

P141

METABOLOMIC ALTERATIONS PREGNANCY-RELATED IN PATIENTS AFFECTED BY MULTIPLE SCLEROSIS

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BACKGROUND-AIM

Pregnancy is a protective condition against autoimmune disorders such as Multiple Sclerosis (MS)(1). Several clinical and MRI predictors of disease reactivation in post-partum have been studied, but laboratory biomarkers have been less investigated (2). Metabolomics could offer a quick and promising approach to finding potential biomarkers and revealing altered biochemical pathways involved in the modulation of the MS (3,4).

METHODS

Serum samples of women with MS and healthy controls (HC) during fertile life, pregnancy, and puerperium were collected. Samples were analyzed through Nuclear Magnetic Resonance (NMR). Multivariate and univariate statistical analyses were performed together with the pathways analysis.

RESULTS

Significant metabolic differences were found when HC samples collected during the fertile life, pregnancy, and puerperium were compared. The most altered pathways were those implicated in the biosynthesis activity, oxidative stress, energetic pathways, and aminoacids metabolism (e.g glutamate, aspartate and glycine metabolism). Similar findings were achieved when the MS samples during the three different phases were compared. Subsequently, a comparison between the HC and MS patients at the different phase was explored. Interestingly, only the model resulting from the comparison of the fertile life phase showed a significant difference ($p<0.0001$).

CONCLUSIONS

Comparable results were obtained between the HC and the MS patients separately during fertile life, pregnancy, and puerperium. From the metabolic point of view, a significant difference was observed only when the two groups were compared at the fertile life phase. The metabolic similarity of the two groups of patients resulted during the pregnancy and the puerperium periods despite the pathological condition suggesting that changes related to the presence of the fetus prevail in the MS features.
The protective role of pregnancy has been confirmed also through the metabolomic approach which once again reveals its potential in the search for biomarkers and for elucidating aetiopathogenic mechanisms which could be useful in the development of novel therapeutics targets for neuroinflammatory and neurodegenerative conditions.

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

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common and aggressive pediatric brain malignancy. 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 genetically understood. SHH-MB is characterized by genetic alterations in key components of SHH signaling, a developmental pathway emerged as an attractive therapeutic target for MB treatment. However, the molecular circuitries governing SHH-MB remain unclear. Recently, we identified INF2, a formin involved in the regulation of actin and cytoskeletal dynamics, as a negative regulator of SHH signaling that deserves further investigation regarding its involvement in MB tumorigenesis.

METHODS

SHH-dependent luciferase functional assay has been performed to evaluate the effect of INF2 overexpression on Gli1 transcriptional activity. We determined INF2 protein and mRNA expression levels in mouse and human SHH-MB samples by immunoblot and qPCR analysis, respectively. The effect of INF2 overexpression on the proliferation of primary SHH-MB cells has been determined by IncuCyte® Live Cell technology. Ubiquitylation and cycloheximide assays have been performed to define proteosome degradation and protein stability of INF2.

RESULTS

We found that INF2 counteracts the transcriptional activity of GLI1, the final and most powerful effector of SHH signaling. INF2 protein levels, but not mRNA, were strongly reduced in murine and human SHH-MBs, suggesting a regulation of INF2 at post- translational level. Indeed, we observed that FBXW7, an E3 ligase and tumor suppressor highly mutated in MB, promotes the ubiquitylation of INF2 and its protein stability, suggesting that the loss of INF2 expression, due to mutations of FBXW7, might play a key role in SHH-MB tumorigenesis. Importantly, we showed that the overexpression of INF2 in SHH-MB primary cells repressed tumor cell proliferation and this correlated with the decrease of SHH signature

CONCLUSIONS

Overall, these findings support a negative role of INF2 in the control of SHH signaling and SHH-dependent MB growth and could further illuminate on the role of cytoskeleton in SHH-dependent cancers and explain as to why mutations of FBXW7 are found in SHH-MB.

P143

BREAST CANCER CELL/ADIPOCYTE CROSSTALK AND TAMOXIFEN RESISTANCE: A POTENTIAL ROLE FOR TXNIP TUMOR SUPPRESSOR GENE

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BACKGROUND-AIM

Despite highly effective, "de novo" or acquired resistance to endocrine therapy is a major clinical concern. Studies suggest that obesity, in addition to promoting BC development and progression, represents a key driver of BC endocrine resistance, but the influence of adipocytes and their secretome on these mechanisms has not properly been investigated.

METHODS

Adipocyte impact on Tamoxifen resistance (TamR) was assessed in co-cultures with differentiated 3T3-L1 adipocytes, a recognized model of white adipocytes, and Tam-resistant (TR) and Tam-sensitive MCF-7 BC cells. Anchorage-dependent/independent growth, wound healing, transmigration and invasion assays were performed. Transcriptomes of adipocyte-derived conditioned media (CM)-treated BC cells were compared by RNA sequencing. TXNIP expression was restored by the histone methyltransferase EZH2 inhibitor DZNEP, the histone deacetylase inhibitor SAHA or lentiviral vector transduction. Kaplan-Meier survival analysis using TCGA dataset was conducted.

RESULTS

Incubation of MCF-7 cells with adipocyte-CM reduced their sensitivity to the inhibitory effects of Tam on growth, motility and invasion. The proliferative, motile and invasive behaviour of TR cells was dramatically increased by adipocyte-CM, further suggesting a supportive role of adipocyte secretome in the development and progression of TR phenotypes. Transcriptomics of MCF-7 and TR cells revealed several differentially expressed genes (FDR $\leq$ 0.05) in response to adipocyte-CM. Among these modifications, a common significant down-regulated gene was Thioredoxin-interacting protein, a key tumor suppressor gene participating in metabolism and oxidative stress. TXNIP downregulation was confirmed by qRTPCR and immunoblotting. Accordingly, restoring TXNIP expression by pharmacological and genetic approaches reversed adipocyte-induced TamR. These results correlated with retrospective analyses showing a statistically significant poorer survival in low TXNIP-expressing BC patients treated with Tam.

CONCLUSIONS

These data shed new light on adipocyte/mammary cancer cell crosstalk working likely in TamR development, suggesting the possibility to target TXNIP function to block this harmful dialogue, especially in endocrine-resistant settings.

P144

GLUTAMINASE-1 INHIBITOR CB-839 INDUCES METABOLOMIC ALTERATIONS IN COLORECTAL CANCER CELLS

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BACKGROUND-AIM

Glutamine (GLN) is considered a “conditionally essential” amino acid, providing building blocks and energy for high proliferating cells, including colorectal cancer (CRC) cells. GLN deprivation induces bioenergetic changes in tumoral cells, resulting in proliferation arrest or adaptation. Therefore, targeting GLN metabolism could represent a winning strategy in anticancer therapy. In clinical trials, CB-839 represents a selective and orally bioavailable Glutaminase-1 (GLS1) inhibitor, thereby avoiding GLN utilization and resulting in antiproliferative effects in several solid and hematological malignancies. To date, poor information about CB-839 effects on the metabolism of CRC cells is available. In this regard, metabolomics offers a promising tool to investigate the metabolic effects underlying the antitumoral activity of this novel therapeutic approach.

METHODS

In this work, four CRC cell lines (Caco-2, HCT116, HT29 and SW480) were treated with different doses of CB-839 (0.5-2 μM) in the presence of GLN for 72h. The cytotoxic effect of the drug was evaluated by MTT assay. The growth rate was estimated through growth curves obtained in control and treated condition. Metabolomic analysis was performed with Gas chromatography–Mass Spectrometry followed by Multivariate and Univariate Statistics to identify metabolic changes resulting after drug treatment.

RESULTS

CB-839 exerted a dose-dependent cytotoxic effect in CRC cells. HT29 and HCT116 were more sensitive to the drug compared to Caco-2 and SW480 cells. CB-839 effect was independent of GLN concentration in cell culture medium (2 or 4 mM). Moreover, a significant decrease in growth rate due to GLS1 inhibition was observed, especially in HT29 cells. Metabolomic analysis of intracellular metabolites revealed that CB-839 induced alteration of metabolic profile in all cancer cell lines. Multivariate models reveal differences based on drug treatment and univariate statistical analysis point out significant alterations of metabolite concentrations consistent with GLS1 inhibition. Particularly, TCA cycle intermediates (fumarate, malate, and citrate) and glutamate content were significantly modified.

CONCLUSIONS

Altogether our results support the potential efficacy of CB-839 in CRC and lay the basis to clarify its metabolic effects.

P145

HYPOXIA-DRIVEN LIPID METABOLISM IN CHRONIC MYELOID LEUKAEMIA CELLS

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BACKGROUND-AIM

The treatment Chronic Myeloid Leukemia (CML) with tyrosine kinase inhibitors (TKI), although very effective in keeping disease under control, rarely results in CML cure. To aim at such an outcome, it is necessary to identify novel molecular targets to be exploited therapeutically in order to hit TKI-resistant CML cells. Several studies have suggested that statins can prevent carcinogenesis and improve cancer patients' overall survival. In particular, in vitro studies showed additive cytotoxic effects against CML cells when statins are administered in combination with TKI. On the basis of these findings, we plan to investigate lipid synthesis pathways as potential targets for CML therapy.

METHODS

CML cell lines were incubated under glucose and/or glutamine deprivation, in normoxic (21% O2) or hypoxic (0.1% O2) conditions. ATP production was measured via a luminometric assay and mitochondrial membrane potential ($\Delta\psi m$) by flow cytometry. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using a Seahorse XF96 metabolic analyzer. Intracellular lipid droplets (LD) were observed via transmission electron microscopy and Nile Red staining. BCR/Abl expression was assessed by immunoblotting. Etomoxir and cerulenin were used to inhibit fatty acid synthesis and oxidation, respectively.

RESULTS

We observed that glutamine plays a major role in ATP production in normoxia, whereas glucose is the main ATP source in hypoxia, allowing CML cell growth in this condition. Under hypoxia, glutamine fuels cells' glycolytic capacity and inhibits basal and maximal respiration. Moreover, we observed an increase of LD in the presence of glutamine in hypoxia compared to glutamine deprivation both in hypoxia and normoxia. A close correlation emerged between lipid metabolism and BCR/Abl expression.

CONCLUSIONS

Our results suggest that in hypoxia glutamine is capable to divert cellular metabolism towards lipid synthesis. This may help cancer cells to survive under serious nutrient restrictions. Based on these and previous results, the co-treatment with TKI and lipid synthesis inhibitors may lead to the reduction/suppression of minimal residual disease in CML patients.

P146

BISPHENOL-A UPREGULATES PREP1 AND PBX1 AND INDUCES INFLAMMATION IN SVF CELLS DERIVED FROM SUBCUTANEOUS ADIPOSE TISSUE OF SEVERELY OBESE INSULIN-RESISTANT AND DIABETIC INDIVIDUALS

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BACKGROUND-AIM

Bisphenol A (BPA) is an organic synthetic compound belonging to the group of the endocrine-disrupting chemicals as it can accumulate and compromise different tissues, such as the white adipose tissue (WAT). the dramatic prevalance of metabolic disorders, such as type 2 diabetes and obesity, has gained increasing attention. These conditions are caused by the complex interaction between genetic susceptibility and environmental factors. Prep1 is a homeodomain transcription factor that, by interacting with its cofactors, Pbx1 and p160, impairs metabolism and causes inflammation in cellular and murine models. However, its role in humans is unknown. In this study, we have evaluated the possible involvement of Prep1 and its cofactors on BPA action in subcutaneous adipose tissue .

METHODS

Stromal vascular fraction (SVF) cells were isolated from subcutaneous adipose tissue (SAT) biopsies of three previously characterized groups of severely obese patients (6 biopsies/group): normoglycemic (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D) subjects.

RESULTS

Prep1 and Pbx1 expression increased by 4.8- and 4.4-fold in SAT biopsies from IGT and T2D patients compared to NGT, while p160 did not change. Low dose Bisphenol A (BPA) increased Prep1 levels by 1.1, 4.5- and 2.6-fold in NGT, IGT and T2D patients, respectively. BPA-induced Pbx1 and proinflammatory molecules expression was also higher in IGT and T2D than in NGT individuals, while IL-10 expression was significantly reduced. Interestingly, BPA levels did not change in all patient groups. In contrast, BPA upregulated GPR30 receptor expression by 1.3-, 3.2- and 3.7-fold in NGT, IGT and T2D patients, respectively, and inhibition of GPR30 reverted the effect of BPA on Prep1 and Pbx1 expression.

CONCLUSIONS

In conclusion, this study suggests hyperinsulinemia as an important environmental factor regulating BPA-GPR30-Prep1/Pbx1 signaling and propose an important implication of environmental disruptors, such as BPA, on metabolic syndrome in the regulation of transcription factors, like Prep1.

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INTRATHYMIC LYMPHOSTROMAL INTERACTIONS: ROLE OF NOTCH SIGNALING IN PROLIFERATION AND APOPTOSIS OF T CELL LEUKEMIA CELLS

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BACKGROUND-AIM

Acute T-cell lymphoblastic leukemia (T-ALL) is a childhood cancer that originates in the thymus and frequently involves bone marrow infiltration. Aberrant Notch signaling has been implicated in the pathogenesis of T-ALL. Many T-ALL cases bear somatic gain-of-function mutations in Notch1, as well as overexpression of Notch3. In addition, it has been shown that a subset of T-ALL shows activating mutations of Notch3. The partnership between Notch and CXCR4 enhances thymic egress and bone marrow infiltration of malignant lymphoblasts to sustain T-ALL maintenance. A major challenge in the field is understanding the underlying mechanisms by which leukemic cells shape the tumor microenvironment to increase their ability to infiltrate and survive within.

METHODS

We performed in vitro cultures of the human T-ALL cell lines, TALL1 and Jurkat, and of primary human thymic epithelial cells (hTECs) derived from the healthy thymus of children undergoing cardiac surgery. Subsequently, cell-free conditioned media from the individual cell cultures were used to cross-cultivate hTECs or the T-ALL cell lines. Through different experimental approaches (FACS, Western Blot, and RNA-Seq), we evaluated the expression and activation of cell surface proteins and T-ALL and hTEC proliferation and survival.

RESULTS

In the above-described culture condition, in which Notch signaling may modulate lymphostromal cells crosstalk, we analyzed: i) the molecular mechanism(s) by which the hTEC conditioned media influence proliferation, viability, and cell surface markers of the leukemic cells, and ii) the effect of leukemia cell conditioned media on the differentiation and survival of hTECs. We found that the hTEC-conditioned media favored TALL1 cells proliferation, whereas it induced cell cycle arrest of Jurkat cells. In addition, we observed that TALL1 and Jurkat conditioned media influenced Notch/CXCR4 and Notch/Jagged1 signaling and viability of hTECs.

CONCLUSIONS

Our study shed light on lympho-stromal cell interaction in the leukemia intrathymic microenvironment and proposes an innovative culture system to test new pharmacological treatments for T-ALL.

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THE POTENTIAL ROLE OF INFLAMMAGING AS MODULATOR FOR RENAL CELL CARCINOMA PROGRESSION

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BACKGROUND-AIM

Chronic, low-grade inflammation and cell senescence, also named Inflammaging, seems to have ambivalent effects in tumor progression. Senescent cells can affect the tumor microenvironment via the senescence-associated secretory phenotype (SASP). Here, we explored the role of inflammaging in the context of Renal Cell Carcinoma (RCC).

METHODS

The expression of pro-inflammatory (PTX3) and SASP-related proteins (p21/ CIP1/WAF1, p16/INK4a, IL-6) in the peritumoral (periRCC) and tumoral (RCC) area of renal samples from 10 patients undergone radical nephrectomy for RCC was evaluated by confocal microscopy. Moreover, a western blotting analysis on normal renal proximal tubular epithelial cells (RPTECs) under hypoxia conditions and in RCC cell lines was conducted.

RESULTS

We observed an increased expression of PTX3 and IL-6 in both periRCC and RCC tissues, as compared to normal renal tissues ($p<0.001$). As of the expression of cell cycle inhibitors, we observed an increase of p21 and p16 levels from normal to periRCC ($p <0.001$) but their reduction in RCC tissues ($p <0.05$).
We subsequently classified tissues according to Fuhrman Grading and we observed higher levels of PTX3 and IL-6 and lower level of cell cycle inhibitors in high grade (G3-G4) than in low-grade tumors (G1-G2), both in periRCC and RCC tissues.
The analysis in vitro showed that after induction of 1% hypoxia PTX3 with SASP-related proteins resulted significantly and progressively increased in RPTEC in a time-dependent manner ($p<0.01$). On the contrary, RCC cell lines showed higher levels of PTX3 and IL-6, as compared to RPTEC at baseline ($p<0.01$ and $p<0,05$, respectively), while cell-cycle inhibitor p21 returned to basal condition and p16 was undetectable ($p<0,01$).

CONCLUSIONS

These preliminary data suggest that inflammaging can affect RCC microenvironment. The downregulation of cell cycle inhibitors and the increased expression of pro-inflammatory factors appear to be crucial for tumor progression and prognostic risk of the disease.

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A NEW FUNCTION OF THE MRN COMPLEX IN THE REGULATION OF CILIOGENESIS

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BACKGROUND-AIM

Microcephaly is caused by the depletion of neuronal progenitors. It is usually associated with defects in both DNA Damage Response (DDR) and centrosome proteins. Curiously, many DDR proteins localize at the centrosome suggesting a functional link between centrosomes and DDR proteins that seems to converge in the control of the neuronal progenitors expansion. The MRN (MRE11/RAD50/NBS1) complex is essential for the activation of the DNA Damage Response (DDR) and hypomorphic mutations in each component of the complex cause DDR defective syndromes characterized by microcephaly. We recently demonstrated that NBS1 depletion increases the length and alters the morphology of the primary cilium (PC) and correlates with a strong down-regulation of the Sonic Hedgehog (SHH) pathway in animal and primary cell models. Since NBS1 localizes at the centrosome and controls its duplication and MRE11 mutations (i.e., p.R633X) may lead to a Nephronophthisis-related ciliopathy, we were prompted to investigate on the possible role for the MRN complex at the centrosome/PC.

METHODS

We used cycling or starved human RPE-1 cells to analyze MRE11 and NBS1 centrosome localization and for studying PC following MRE11 depletion. Moreover, we evaluated PC length and the frequency of ciliated cells in murine primary neuronal cultures and human RPE-1 cells following exposure to clastogenic drugs.

RESULTS

MRE11 localizes at the centrosome in different phases of the cell cycle and both NBS1 and MRE11 independently localize at the base of the PC (basal body), since RNAi of each component does not prevent proper localization of the other one. Moreover, similar to NBS1 KO, MRE11 depletion or its pharmacological inhibition via mirin induces an elongated and dysmorphic PC. On the contrary, genotoxic drugs do not significantly affect PC length and morphology, implying that the observed ciliary phenotypes are not a general consequence of DNA damage or DDR activation.

CONCLUSIONS

Our data indicate that, besides its canonical function in the DDR, the MRN complex exerts a function at the PC, which might be relevant for a better understanding of the molecular mechanisms underpinning the neurological phenotypes of MRN dysfunction-related human disorders

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UNRAVELING THE ROLE OF UNFOLDED PROTEIN RESPONSE (UPR) IN MEDULLOBLASTOMA CANCER STEM CELLS (MBCSS)

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common malignant infant brain tumor. The clinical approach consists of multimodal strategies with debilitating neurological sequelae and often tumor relapse. Medulloblastoma stem cells (MBSCs) are a fraction of tumor cells with high proliferation potential and able to adapt to restrictive conditions in tumor milieu, thus driving the resistance to therapy. Unfolded Protein Response (UPR) players were found over-expressed in tumors of different tissue-origin correlating with poor prognosis and low patient survival. UPR is a mechanism that restore proteostasis in endoplasmic reticulum (ER) by activating the sensors IRE1, ATF6 and PERK to coordinate a cytoprotective response. UPR sustains cancer cells progression and promotes resistance to chemotherapy. Also, in some solid tumors UPR modulates stem properties. However, little is known about the role of UPR in MB. Thus, the aim of this work is to delve into the role of UPR in MBSCs.

METHODS

Human group 3 MB cell lines (CHLA-01, D283 and D341-Med) were grown in stem selective medium (B27™) for 72hrs. Then, cells were treated with ER stress inducers Brefeldin A 0,3uM and ONC201 4uM for 24hrs. Upon treatment, clonogenic assay was conducted. UPR players ATF4/CHOP and stemness markers were analysed by WB and confocal microscopy.

RESULTS

By investigating the expression and activation of UPR players in MBSCs, we discovered, even in resting conditions, a preferential activation of the UPR PERK branch in MB cells grown in stem-like condition. Upon treatment with Brefeldin A and ONC201, an induction of cell death and an increase of ATF4 expression in the nucleus was observed. The treatment reduced clonogenic properties and likely disrupts tumor sphere integrity. Furthermore, we observed a modulation of the stemness markers CD133 and OCT4, in terms of post-transcriptional modifications.

CONCLUSIONS

These results suggest that over-activation of UPR may affect MBSCs by a dual mechanism. Firstly, UPR activation triggers apoptosis and impairs self-renewal, tumor sphere forming capability, hence globally reducing the undifferentiated compartment in MB tumor bulk. Our results shed light on the basic biology of the UPR in MBSCs and suggest a proof of principle for the design of novel therapeutics.

P151

EXTRACELLULAR VESICLES RELEASED FROM LEPTIN-TREATED BREAST CANCER CELLS SUSTAIN CELL METABOLISM

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BACKGROUND-AIM

Extracellular vesicles (EVs) have been recognized key mediators of cell-to-cell communication in breast cancer (BC). The secretion of EVs and selection of their molecular cargo are highly regulated processes. We have previously demonstrated that the adipokine leptin, whose circulating levels correlate with adipose tissue mass, is an inducer of EVs' release in BC cells. Preliminary data, obtained by LC-MS/MS analysis, showed a specific proteomic signature, enriched in mitochondrial components in EVs released by MCF-7 BC cells treated with leptin (Lep-EVs). We investigated the potential role of these EVs in modulating metabolism of BC cells and of macrophages, the most abundant immunological component in tumor microenvironment.

METHODS

EVs have been isolated from ER-α positive MCF-7 BC cells by using ultracentrifugation method and characterized by following MISEV guidelines. EV impact on MCF-7 cells energy production and mitochondrial function was assessed by ATP, MitoTracker Deep Red/Orange assays. MCF-7 and THP-1 cell metabolic profile was analyzed by Mito Stress and Glycolysis Stress test performed by Seahorse XFe96 analyzer. Functional assays in MCF-7 cells (i.e. migration/invasion) and in macrophages (i.e. polarization/phagocytosis assay) were performed after Lep-EV treatment.

RESULTS

We observed that Lep-EVs significantly enhanced the amount of ATP produced in MCF-7 cells (2 fold over C-EVs), as well as the ratio of mitochondrial membrane potential/mass. Moreover, Lep-EVs increased cellular bioenergetics in MCF-7 cells as measured by oxygen consumption rate along with a higher basal and maximal respiration compared to C-EVs. In addition, we revealed that the message carried by Lep-EVs sustained the polarization of M0 macrophages into M2-like tumor associated macrophages, in terms of metabolic features and phagocytic activity.

CONCLUSIONS

Overall, our results indicate that leptin by inducing the release of EV-enriched in mitochondrial proteins may control metabolism of breast cancer cells as well as of macrophages. Characterization of tumor-derived EV protein cargo might allow to identify unique features and specific metabolic mechanisms useful to develop novel therapeutic approaches for treatment of BC, especially in obese condition.

P152

IDENTIFICATION AND VALIDATION OF DNA METHYLATION HOTSPOTS AS BIOMARKERS FOR CUTANEOUS MELANOMA

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BACKGROUND-AIM

Several studies have demonstrated that the development of cutaneous melanoma (CM) is not only prompted by gene mutations, but it is also due to epigenetic modifications. Among these, DNA methylation represents an early event of neoplastic transformation associated with the dysregulation of genes involved in apoptotic processes and cell proliferation.
On these bases, the aim of the study was to evaluate the methylation status in CM in order to identify and validate DNA methylation hotspots (methDNA) involved in the development of CM.

METHODS

For these purposes, a bioinformatics analysis was performed using the EpiMethEx R-package to evaluate the correlation between DNA methylation and gene expression data contained in TCGA and GTEx databases. Through further computational analyses, a set of methDNA affecting genes involved in the development and progression of CM was identified. Among these genes, methDNA affecting RARB and ISL1 were selected for the validation analyses performed on melanoma cell lines and FFPE samples by using a custom protocol defined Methylation-Sensitive Restriction Enzyme-ddPCR (MSRE-ddPCR) assay.

RESULTS

Through EpiMethEx, two (TSS200 and EX1) and three (isl28, isl53 and EX1) hotspots were identified for RARB and ISL1, respectively. The expression and methylation levels of these hotspots were evaluated in 5 CM cell lines by using MSRE-ddPCR demonstrating the negative correlation existing between RARB promoter methylation and its expression and the positive correlation existing between ISL1 intragenic methylation and its expression. Such correlations were also validated by demethylating the cells through 5-azacytidine.
These in vitro results were confirmed on FFPE melanoma samples and normal nevi. In particular, the MSRE-ddPCR analyses demonstrated the hypermethylation of the TSS200 and EX1 methDNA of RARB in CM samples. Similarly, the isl28 hotspot of ISL1 was also hypermethylated in cases compared to controls.

CONCLUSIONS

The results obtained demonstrated the high predictive value of EpiMethEx investigations and the accuracy of the MSRE-ddPCR protocol. These results encourage the development of novel diagnostic strategies based on the analysis of epigenetic biomarkers in CM.

P153

EPIGENETIC MODULATION OF MICRORNAS AND TUMOR PROMOTING PROTEINS IN BREAST CANCER: RESULTS FROM THE DEDICA CLINICAL TRIAL

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BACKGROUND-AIM

Recent studies have demonstrated that healthy diet and physical activity can improve the prognosis of breast cancer (BC) patients and their quality of life through epigenetic modifications induced by lifestyle interventions; however, the precise molecular mechanisms behind these positive effects have not been identified yet.
On these bases, we proposed a clinical trial based on the administration of high- or low-intensity low-glycemic index diet, exercise and vitamin D (DEDiCa study) to evaluate if lifestyle interventions may improve the prognosis of BC patients and induce positive modifications on microRNAs (miRNAs) and protein markers.

METHODS

A set of miRNAs associated with BC and modulated by diet and exercise was identified through computational analyses performed on GEO DataSets miRNA expression data. The expression levels of the selected miRNAs were investigated by ddPCR in liquid biopsy samples obtained from 506 DEDiCa BC patients at different time points (baseline, 12 and 33 months) to evaluate positive epigenetic modifications induced by the interventions proposed in the trial. The protein levels of IGF1 and IGFBP3, as markers of BC aggressiveness modulated by miRNAs, were also analyzed through ELISA assays to further evaluate the beneficial effects of lifestyle interventions.

RESULTS

The computational analyses allowed the selection of five miRNAs, miR-125b-5p, miR-486-5p, miR-21-5p, miR-139-5p and miR-7-5p, as markers of response to lifestyle treatments.
The ddPCR analyses demonstrated that the expression levels of the two tumor suppressor miRNAs miR-125b-5p and miR-486-5p were significantly increased after dietary and exercise interventions in both groups. The increment of these two miRNAs was also associated with the decrement of the protein levels of both IGF1 and his transport protein IGFBP3 which was more evident in the group of patients with high-intensity interventions.

CONCLUSIONS

The results revealed that lifestyle interventions induce positive modifications in both miRNA expression levels and tumor-related proteins encouraging the adoption of healthy diet and exercise as supportive treatments for BC patients.

P154

DIFFERENTIAL EXPRESSION OF THE MITOCHONDRIAL PROTEASE LONP1 ISOFORMS IN CANCER

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BACKGROUND-AIM

Lonp1 is a mitochondrial protease that degrades oxidized and damaged proteins, assists protein folding, and contributes to maintain mitochondrial DNA (mtDNA). The human full-length form of Lonp1 (ISO1) is formed by 959 aa. Two other isoforms are present in humans, resulting from alternative splicing: Isoform-2 (ISO2) is 895-aa long and lacks aa 42-105, isoform-3 (ISO3) contains 763 aa and lacks aa 1-196, including the Mitochondrial Targeting Sequence (MTS). Lonp1 is upregulated in several type of cancer; its higher expression has been associated with higher tumour aggressiveness. Accordingly, we aimed at determining if and how higher tumour aggressiveness is associated with a particular isoform of Lonp1.

METHODS

Transcription data of different forms of cancer and normal mucosa have been obtained by TSV database (TSVdb). mtDNA has been quantified by droplet digital PCR; respiration has been analyzed by using Mitrostress assay kit on a Seahorse analyser XF96; protein levels were evaluated by Western blot and intracellular distribution of isoforms by confocal microscopy.

RESULTS

We analysed the expression of Lonp1 splicing variants in different primary solid tumours and normal tissue counterpart in the public database TSVdb. ISO1 was upregulated in lung, bladder, prostate and breast cancer, and ISO2 in all the other types (rectum, colon, cervical, bladder, prostate, breast, head and neck). ISO3 did not show significant changes. We overexpressed ISO1, ISO2 and ISO3 in colorectal cancer cells SW620. ISO1 isoform is expressed exclusively in the mitochondria, ISO2 is present in the organelle and in the cytoplasm, and ISO3 exclusively in the cytoplasm. Overexpression of ISO3 increased mtDNA copy number/cell. Overexpression of ISO1 determined a reduction in respiration and a tendency to switch to glycolysis. All isoforms increased ROS levels, but this was particularly evident with ISO2 and ISO3. Overexpression of ISO1 and ISO2, but not ISO3, determined un upregulation of EMT related proteins, such N-Cadherin. Interestingly, ISO2 and ISO3 causes this change likely through different pathways, as ISO1 overexpression determined a downregulation of Snail1 but not Snail2, while ISO2 has the opposite effect.

CONCLUSIONS

Three main isoforms of Lonp1 coexist in the cells, that localize in different cell compartments. Their expression is different in different cancer types, suggesting a diverse biological role for Lonp1 isoforms in cancer development.

P155

DISSECTING A NOVEL UNCANONICAL ROLE OF THE NIJMEGEN BREAKAGE SYNDROME PROTEIN NBS1 ON THE PRIMARY CILIUM

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BACKGROUND-AIM

NBS1 is a member of the MRE11/RAD50/NBS1 (MRN) complex, and when mutated is responsible for the DDR-defective Nijmegen Breakage Syndrome (NBS). In addition to causing microcephaly and cerebellar hypoplasia, we recently demonstrated that central nervous system (CNS)-restricted NBS1 KO completely abolishes medulloblastoma (MB) insurgence and down-regulates the SHH pathway in a SHH MB-prone mouse model, which suggests an epistasis of NBS1 KO on the SHH pathway. Interestingly, microcephaly is a phenotype common to other DDR-defective syndromes and to dysfunctions in centrosome-related proteins. Due to an emergent link between DDR and centrosome/Primary Cilium (PC) proteins, and to the notion that SHH signaling is dependent on the PC, we raise the hypothesis that NBS1 may contribute to cerebellar development and tumorigenesis by regulating the SHH pathway through a novel role on ciliogenesis.

METHODS

We generated and analyzed new mice models with NBS1 KO restricted to cerebellar Granule Cell Progenitors (GCPs) in both WT and SHH-MB prone backgrounds. We manipulated in vitro primary GCPs from transgenic mice as well as human immortalized cells; we further examined RNA and protein expression by Microfluidic CARD and Western Blot analyses, and PC morphology by confocal microscopy.

RESULTS

We reveal that NBS1 loss in GCPs abrogates Ptch1-dependent MB formation, implying that the NBS1 KO phenotype is dominant on that of Ptch1 loss. Moreover, NBS1 depletion in GCPs is sufficient to impair cerebellar development, leading to reduced cerebellar size with impaired proliferation and premature differentiation of GCPs due to inhibition of the SHH pathway in an in vivo, ex vivo and in vitro context. We further show that NBS1-deficient cells consistently display severe dysmorphisms of the PC in an in vivo, ex vivo and in vitro framework, most commonly reflected in a strikingly elongated phenotype.

CONCLUSIONS

Our results uncover a new function of NBS1 on the regulation of PC/SHH pathway that eventually contributes to cerebellar development and tumorigenesis. Finally, they suggest that PC dysfunction might be the molecular explanation of the neurological phenotypes common to patients with centrosome- and DDR-defective syndromes.

P156

HIGH GLUCOSE AND SENESCENCE CONDITIONS INDUCE AN INCREASED BURDEN OF CYTOSOLIC NUCLEIC ACIDS, UNBALANCING THE ANTI-VIRAL AND PRO-INFLAMMATORY RESPONSES

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BACKGROUND-AIM

Increasing evidences highlighted an association between cytosolic nucleic acids accumulation and the activation of the DNA Damage Response (DDR). Endogenous dsRNA, dsDNA and DNA:RNA hybrids can trigger and sustain an inflammatory loop inducing proinflammatory cytokines and type I interferons. Here, we aimed to explore whether senescence and/or high glucose conditions can modulate the accumulation of cytoplasmic nucleic acids and the release of molecules with anti-viral (IFN type 1) or with pro-inflammatory (IL-1β, IL-6, IL-8) activity.

METHODS

HUVECs were cultured until replicative senescence. Non-senescent (CTR) and senescent (SEN) cells were than cultured for 7 days under normal (5.5 mM, NG) or high (25 mM, HG) glucose concentrations. Classical biomarkers of cellular senescence, such as proliferation rate, pro-inflammatory cytokines expression, telomere length and cytosolic nucleic acids quantified by immunofluorescence, were analyzed in all conditions.

RESULTS

An increased expression of IL-1β, IL-6, IL-8, a significant reduction of telomere length (TL) and an increased amount of cytosolic dsRNA and dsDNA were observed in SEN cells compared to CTR ones. The same effects were observed in both CTR and SEN cells cultured in high glucose condition, demonstrating that high glucose exposure can induce a senescent-like phenotype.
The accumulation of endogenous nucleic acids leads to a shift from an antiviral response to a pro-inflammatory response. Notably, the stimulation with exogenous dsRNA promotes an antiviral response.

CONCLUSIONS

Human endothelial senescent and/or high glucose exposed cells shift their secretome from antiviral to proinflammatory profile in association with an accumulation of endogenous cytoplasmic nucleic acids. This could contribute to explain the association between high glucose exposure of endothelial cells and the increased systemic pro-inflammatory status.

P157

EXPLOITING THE STING PATHWAY TO CONVERT COLD MYCN AMPLIFIED NEUROBLASTOMA INTO INFLAMED AND IMMUNOTHERAPY-RESPONSIVE DISEASES

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BACKGROUND-AIM

MYCN deregulation drives several neoplasia, including MYCN amplified neuroblastoma (MNA NB). It associates with aggressive behavior and refractoriness to standard therapy, raising the need for more effective interventions.
The STING pathway has gained interest in cancer immunotherapy due to its ability to stimulate type-I interferon (I-IFN) transcription and immune/inflammatory responses against tumor cells, by sensing cytosolic DNA derived from viral or endogenous sources (damaged DNA from nucleus or mithocondria).
Whilst MYCN-driven tumors are characterized by high levels of oncogene-derived DNA damage, they are typically associated with a ‘cold’ phenotype, that is lack of tumor infiltrating leukocytes, low I-IFN transcriptomics and chemokine expression, suggesting that the STING pathway is attenuated in these tumors.
However, the status of the STING pathway and whether it could be exploited for the treatment of MYCN-driven tumors has remained so far unexplored.

METHODS

A panel of non-MNA and MNA NB cell lines and MYCN-inducible models were used. The expression and activation of the STING pathway were evaluated by WB and qPCR. ChIP assays as well as treatments with 5-aza-2’-deoxycytidine (DAC) were performed to assess the epigenetic state of the STING pathway. STING agonist (cGAMP/diABZI) and other STING pathway inducers (dsDNA90/olaparib) were used.

RESULTS

We demonstrate that MNA NB cells express undetectable levels of cGAS and STING mRNA. Coherently, STING pathway activation is impaired in these cells. Inducible MYCN expression led to cGAS/STING repression, supporting the idea that MYCN enforces the suppression of the pathway. Mechanistically, the cGAS/STING repression occurs through epigenetic silencing, including DNA methylation and repressive histone post-translational modifications (H3K27me3, H3K9me2/me3). Restoring cGAS/STING by transgene expression or by DAC-induced derepression is sufficient to confer responsiveness to STING pathway inducers in MNA NBs.

CONCLUSIONS

In conclusion, our data reveal that STING pathway is silenced in MNA NB through epigenetic mechanisms enforced by MYCN. Understanding and counteracting these mechanisms may provide new therapeutic strategies for MYCN-driven tumors based on STING pathway reactivation.

P158

TARGETING OF DRP1, THE MASTER REGULATOR OF MITOCHONDRIAL FISSION, INHIBITS MULTIPLE MYELOMA LIPOGENESIS TO TRIGGER FERROPTOSIS

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BACKGROUND-AIM

Mitochondrial dynamics, a process finely regulating mitochondria shape and number through continuous cycles of fission and fusion, has been found associated with several cancer hallmarks. Herein, we investigated, for the first time, its expression pattern and functional significance in multiple myeloma (MM).

METHODS

Proeasome inhibitors (PI)-resistant cell lines were generated by stepwise exposure of increasing concentrations of PI. Mitochondrial structure was assessed by TEM. Cell viability was assessed by Cell Titer Glo or CCK8 assays. ROS were determined by H2DCFDA, mitochondrial superoxide species by MITOSOX red staining. Lipid peroxides were assessed by BODIPY C11 FACS-analysis. DNM1L mRNA expression was obtained by datasets GSE5900, GSE13591 and GSE2658.

RESULTS

The DNM1L gene encoding the GTPase Drp1, i.e. the master regulator of mitochondrial fission, was found upregulated in MM plasma cells from three different GEP datasets, as well as in a panel of 11 MM cell lines. MM cells, especially those resistant to PI, displayed upregulation of the main fission effectors, namely Drp1, its active S616 phosphorylated form, the Drp1 receptor MFF. TEM analysis revealead rearrangements suggestive of ongoing mitochondrial fission in primary CD138+ MM cells as well as in MM cell lines, especially in the PI-resistant cell lines or in plasma cells obtained from RRMM patients. Pharmacological targeting of mitochondrial fission with a novel Drp1 inhibitor reduced MM cell viability, even in co-culture with bone marrow stromal cells, and increased ROS and mitochondrial superoxide species, while sparing healthy PBMCs. At the molecular level, pharmacological or genetic silencing of Drp1 decreased the expression of MYC and SREBF transcription factors, leading to: down-regulation of lipogenesis-related genes (i.e. SCD1, ACSL1), raise in lipid peroxides and induction of ferroptosis, also supported by down-regulation of GPX4 and SLC7A11.

CONCLUSIONS

These data shed light on dysregulated mtochondrial dynamics, and pave the way to the use of Drp1 inhibitors for therapeutic induction of ferroptosis in MM.

P159

EVALUATION OF THE THERAPEUTIC EFFICACY OF A BIFUNCTIONAL ANTIBODY IN COMBINATION WITH CHEMOTHERAPEUTIC DRUGS, IN A MOUSE MODEL OF PANCREATIC ADENOCARCINOMA (PDAC) GENERATED BY ECO-GUIDED IMAGING.

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BACKGROUND-AIM

PDAC is one of the cancers with worst prognosis1,2. This occurs because PDAC is often diagnosed at advanced stages, and because of an intrinsic resistance to the most common chemotherapeutic drugs. Advanced stage PDAC is treated primarily with gemcitabine3, known to be metabolically unstable. To compensate, it is administered in high doses, which generates serious side effects. To overcome these problems, we generated an orthotopic xenograft mouse model in which we tested the antineoplastic efficacy of a new engineered bifunctional antibody (scDb, Patented by MCK therapeutics, n°: PCT102017000083637) which has been proven to affinity target PDAC cancers, in combination with Gemcitabine (GEM).

METHODS

The orthotopic PDAC mouse model was developed by USGI of 1 X 106 PANC-1 cells on the pancreas of athymic nude mice. A 20 µL bolus of cells suspended in PBS was injected directly into the pancreas using Hamilton syringe with a 27 g needle previously placed in the mechanical syringe holder and lined up parallel to the US-transducer and perpendicular to the body. VevoLAZR-X system was used for USGI and for monitoring the tumor development. 4 groups of treatment were made: 1) CTRL (saline); 2) gemcitabine 0.5 mg/mouse; 3) scDb (bifunctional antibody directed against the molecular target hERG1/β1 integrin complex) 320 mg/mouse;4) gemcitabine 0.1 mg/mouse + scDb 320 mg/mouse. Gemcitabine was administered i.p. 3 times per week; scDb was administered i.v. every day.

RESULTS

The development of the PDAC has been characterized and monitored by US imaging. The treatment started after 2 weeks from the cell injection and continued for the next 3 weeks. In terms of tumor growth, we observed a similar effect between scDb and gemcitabine at the lower dose (subtherapeutic), but the most relevant result was obtained in the group treated with scDb+gemcitabine 0.1 mg/mouse. A similar reduction of tumor growth was indeed observed in the group treated with the combination scDb+gemcitabine and the group treated with gemcitabine 0.5 mg/mouse.

CONCLUSIONS

The orthotopic PDAC model, derived from US-guided injection, showed a slower growth rate of tumor developed that is preferable because allows to better monitor a therapeutic effect over time. In this preliminary set of experiment was observed an interesting antitumoral effect of the combination therapy scDb + gemcitabine. This allows to reduce the dose of the gemcitabine maintaining the anti-neoplastic effect overcoming the side effects of chemotherapy.

P160

UNVEILING CARDIAC IL-33/ST2L PATHWAY DYSREGULATION IN A RAT ANIMAL MODEL OF OBESITY

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BACKGROUND-AIM

IL33/ST2L pathway exerts a fundamental protective role in cardiac tissue. Conversely, the soluble form of ST2 receptor (sST2) is a decoy receptor, which blocks IL33 signaling. On the other hand, IL-33 overproduction is also related to pro-inflammatory signals in asthma, allergies and sepsis. Interestingly, IL33/ST2L signaling is involved in fat deposition. Here, we evaluated the influence of adipose tissue on IL-33/ST2 pathway regulation and its role in cardiac steatosis and fibrotic remodeling.

METHODS

Ten obese non-diabetic male Zucker rats (OB) and ten lean (L) littermates were sacrificed at 25 weeks of age. H9C2 cells were treated with Ghrelin (GHR 10mM for 72hours).

RESULTS

By coculturing H9C2 cells with visceral adipose tissue derived from L- or OB-rats, we found that IL-33 was increased in cells stimulated with adipose tissue from OB-rats. Conversely, ST2L expression was greatly increased following L-rats stimulation. Finally, Epac1 expression was reduced by fat stimulation, negatively related to ST2L expression. As OB rats present an increase in serum GHR level (due to lack of leptin signaling), we assessed that GHR stimulation in H9C2 cells allowed IL-33 and GHSR expression. In line with previous results, the excess of fat determined increased IL-33 expression in cardiac tissue while ST2L receptor was reduced in Ob-rats more significantly than in L-rats. We also demonstrated reduced Epac1 expression, increase of GHSR and TGFb/collagen deposition - markers of fibrotic remodeling, even if we did not reveal significative remodeling of cardiac architecture

CONCLUSIONS

Besides IL-33/ST2 protective role, IL-33 exerts a less known role of transcription repressor. In this sense both pro- and anti-inflammatory actions have been ascribed to nuclear IL-33. Here we showed that obese adipose tissue promoted IL-33 production both in vivo and in vitro. This pathway leaded to a reduction of ST2L suggesting loss of cardioprotection and a consequent Epac1 reduction. In Zucker rats, GHR up-regulation determines IL-33 increase in cardiac tissue, possibly representing a compensative pathway to counteract the excess of FFA. Since chronic IL-33 overproduction will finally end in maladaptive remodeling, in Zucker rats GHR excess protects the hearth by reducing apoptosis and promoting cardiomyocytes’ survival.

P161

METABOLIC REWIRING IN HUMAN ENDOTHELIAL CELLS EXPOSED TO HIGH GLUCOSE - LESSONS FROM CULTURES IN 3D MICROFLUIDIC CHIPS

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BACKGROUND-AIM

Glucose-induced endothelial dysfunction plays a fundamental role in the development of diabetic vascular complications. We studied the metabolic adaptation of human endothelial cells to an overload of glucose.

METHODS

Primary human endothelial cells isolated from the umbilical vein (HUVEC) were cultured in 2D or in 3D microfluidic chips (shear stress ~ 0.4 Pa) and treated for 24h with physiological (5.5 mM) or two high concentrations (11.1 mM and 30 mM) of D-glucose. L-glucose (30 mM) was used as control of osmolarity. Confocal microscopy and Synchrotron-based Cryo-SXT were utilized.

RESULTS

High glucose 1. upregulated GLUT1, with consequent increase of glucose oxidation in the glycolytic pathway and accumulation of lactate; 2. decreased mitochondrial (mt) membrane potential, induced the release of mtROS and altered mitochondrial dynamics. This aberrant mitochondrial function is reflected by the decreased oxygen consumption rate and reduced ATP content in isolated mitochondria; 3. increased lipogenesis and reduced fatty acid oxidation, resulting in the accumulation of lipid droplets.

CONCLUSIONS

High concentrations of glucose reprogram metabolism in HUVEC. Some differences emerge between cells cultured in static 2D versus microfluidic channels.

P162

MAGNESIUM DEFICIENCY INDUCES LIPID ACCUMULATION IN CULTURED VASCULAR ENDOTHELIAL CELLS VIA OXIDATIVE STRESS

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BACKGROUND-AIM

Magnesium deficiency promotes endothelial dysfunction, thus contributing to atherogenesis. We investigated lipid metabolism in primary human endothelial cells cultured in different concentrations of magnesium.

METHODS

Human endothelial cells were cultured in medium containing from 0.1 to 5 mM magnesium for 24 h. Reactive oxygen species (ROS) were measured by DCFDA. Lipids were detected after staining with O Red Oil or BODIPY 493/503. The levels of EDF-1 and PPARγ were visualized by Western blot.

RESULTS

Magnesium deficiency leads to the accumulation of lipid droplets storing triglycerides. This results from magnesium dependent increase of ROS, which, in turn, upregulates EDF-1, a transcriptional coactivator for non-steroid nuclear receptors involved in lipid metabolism. Magnesium deficiency also increases PPARγ, a nuclear receptor regulating transcription of several genes implicated mainly in fatty acid and energy metabolism.

CONCLUSIONS

Magnesium deficiency induces the deposition of lipids by upregulating PPARγ and EDF-1. Because of the reduced dietary intake, subclinical Mg deficiency is common and associated with increased cardiovascular risk. Therefore, our studies offer novel insights into the complex mechanisms leading to endothelial dysfunction in Mg deficiency.

P163

URINARY LIQUID BIOPSY AS A POTENTIAL SCREENING TOOL FOR THE EARLY DETECTION OF PRE-CANCEROUS AND CANCEROUS CONDITIONS

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BACKGROUND-AIM

Given the non-invasive way to obtain urine and its resourceful, urine liquid biopsy may be a useful source of patient-derived material, especially for kidney, prostate, bladder and upper and lower tract urothelial carcinoma. Cytology involves the microscopic assessment of atypical/malignant cells (A/MTC) shed from the urinary tract. However, cytology is significantly accurate in diagnosing high-grade urothelial cancers, but not for those of low grade. With the advancements in high-through put methods, urinary nucleic acids (NA) can be successfully molecular characterize to reflect patient's clinical status. Biomarkers such as TERT, TP53, EGFR, ERBB2, PIK3CA and FGFR3 could help in the early non-invasive diagnosis of the diseases.

In our study, we hypothesized that the combination of the urinary NA analysis with urine screening (chemical and microscopical) could represent an easily and rapid reflex test for indeterminate samples characterized by atypical cells, early identifying patients likely to develop urogenital disease.

METHODS

22 urine samples with suspicious cells were first analyzed by the iRICELL analyzer (BD) and then characterized by microscopy and NGS. Cytology was performed by an automated Papanicolaou stainer while circulating NA were extracted using the QIAamp Circulating kit. NGS was performed by Illumina Miseq, sequencing the 38 most frequently mutated genes in cancer. Data were interpreted by Andas and annotated using public databases.

RESULTS

In this pilot study, 22 subjects were divided into 2 groups: half had a diagnosis of urothelial/bladder cancer (patients), while the other half not (controls). Morphological atypia was confirmed by urinary cytology of all cancer patients-derived samples (100% agreement). NGS analysis of cfDNA (n=19) revealed a sensitivity of 91% and a specificity of 89%. The most frequent mutated genes were: ERBB2 (45%), PIK3CA and FGFR3 (18%). The microsatellite instability was found in 14% of cases. Further investigations are in progress for the evaluation of mRNA fusions.

CONCLUSIONS

A/MTC, particularly with the phase contrast microscopy, can be detected in fresh unstained urine samples routinely handled for physical, chemical and morphological urine examination. Urine positive for A/MTC cells need further study. A reflex urine NA analysis could be an effective strategy to detect pre-cancerous or cancerous conditions in early stage.

P164

UNRAVELING THE ROLE OF NOTCH2 MEDIATED BY EVS IN BONE MICROENVIRONMENT: FOCUS ON PRE-OSTEOBLAST AND STROMAL CELLS.

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BACKGROUND-AIM

The bone microenvironment in Multiple Myeloma (MM) is crucial in MM oncogenesis. The initial bone disruption affects osteoblastogenesis and could promote MM development. The aberrant overexpression of NOTCH2 and the ligands Jagged1 and 2 in MM cells mediate their pathological communication with BM leading to angiogenesis and osteoclastogenesis. Extracellular vesicles (EVs) have been widely shown to play a pivotal role in MM microenvironment communication by transferring their specific functional molecular cargo. The role of NOTCH pathway on osteoblastogenesis in myeloma bone microenvironment is unknown. Thereby, in this work, we aim to assess the effect of MM-derived EVs on the bone microenvironment considering stroma and preosteoblasts and elucidate the role played by the NOTCH pathway in EV-mediated communication in osteoblastogenesis.

METHODS

The MM cell line OPM2 was forced to express a shRNA for Notch2 (N2-KD), Jagged 1-2 (J1J2-KD) or the scrambled control (SCR) by using a lentiviral vector (pTRIPZ). EVs collected were used to treat stromal cells (HS5) or preosteoblasts (MC3T3-E1). Cell viability and apoptosis was tested. Ostoblastogenic genes profile were be analysed in q-PCR.

RESULTS

EVs from OPM2 wyld type induce cell viability both in HS5 and in MC3T3-E1 after 24-48 hours exposure. EVs from OPM2 SCR, N2-KD and J1J2KD induce cell viability both in HS5 and MC3T3E1. In MC3T3-E1 the EVs from N2-KD show an higher increase than SCR. The apoptosis rate is coherent with the cell viability trend found. We have also investigated the gene expression of Runx2, a key transcription factor involved in osteoblastogenesis. We found an increase of Runx2 gene expression in MC3T3-E1 cells exposed to EVs from N2-KD.

CONCLUSIONS

These findings could be the first piece of the puzzle to demonstrate the role of NOTCH in bone disruption in MMs bone microenvironment.

P165

A PLASMA METABOLOMIC SIGNATURE OF HEAD AND NECK PARAGANGLIOMA

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BACKGROUND-AIM

Paragangliomas (PGLs) and pheochromocytomas (PCs, collectively PPGLs) are neural crest-derived tumors that arise from parasympathetic or sympathetic paraganglia. PCs are characterized by excessive secretion of catecholamines, parasympathetic PGLs, mostly arising in the head and neck region (HNPGLs), are usually-hormone-silent. Here, we investigated the metabolomic imprint of HNPGLs on the patients' plasma.

METHODS

We screened 57 plasma metabolites comprising amino acids, acyl-carnitines, lysophosphatidylcholines and succinylacetone, obtained from 59 HNPGLs and 25 healthy donors as a control group. The screening was performed by flow injection analysis using tandem mass spectrometry (FIA-MS/MS). As a second approach, the metabolites were also analyzed by extraction from dried blood (DBS) and whole blood (WBS) spotted on filter paper according to PerkinElmer's protocol. Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) were performed on SIMCA® 17 Multivariate Data Analysis Software (Sartorius).

RESULTS

Our targeted metabolomics analysis revealed a distinct metabolic signature for HNPGLs, as showed by PCA and PLS-DA clustering analysis. The variables that most affected the modelling included long- and medium-chain acylcarnitines, as well as glutamine (Gln), suggesting an alteration of mitochondrial fatty acid β-oxidation and a possible pivotal role of Gln in PGL metabolism as anaplerotic substrate. Moreover, the comparison with the control group showed statistically significant differences also for metabolites involved in the urea cycle (Ornithine, Arginine). In addition, comparison with a group of head and neck tumors other than HNPGL (n=12) revealed that some of tested markers were specific for HNPGLs.

CONCLUSIONS

Our data highlighted the importance of lysophosphatidylcholines, glutamate, deoxyadenosine and some long-chain acylcarnitines as potential biomarkers of PGL. Furthermore, the metabolomic analysis on dried blood spot could be a powerful screening tool for the easy and non-invasive monitoring of PGL patients.
(RMC acknowledges the support of AIRC IG grant 24501).

P166

CARBOXYLESTERASE 1 (CES1) IS A PROMISING DRUGGABLE TARGET IN OVARIAN CANCER

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BACKGROUND-AIM

Tumour cells show an incredible metabolic plasticity. This flexibility is a key factor that allows cancer cells to cope with the low nutrient concentrations in the tumour microenvironment, thus sustaining their proliferation and metastatic spread. Recently, carboxylesterase 1 (CES1), has been identified in colorectal carcinoma (CRC) as an essential NF-κB-regulated lipase promoting cancer-cell survival and metabolic adaptation under condition of energy stress. Specifically, CES1 was able to promote the survival of CRC cells by increasing TAG breakdown to fuel fatty acid oxidation and preventing their toxic build-up. Like CRC, ovarian carcinoma (OC) preferentially metastasizes to peritoneal cavity and infiltrates the omentum, a fat rich organ, and recent evidence pointed out the involvement of lipid metabolism in energy stress response. Therefore, we investigated whether CES1 could play an important role also in the metabolic adaptation of OC cells.

METHODS

Public datasets of OC patients were analysed. A panel of four OC cell lines were tested at the baseline for CES1 expression and bioenergetic parameters by qRT-PCR, Western blot (WB) and Seahorse XFe96. Changes in CES1 levels, metabolic phenotype, autophagy flux and survival under energy stress conditions with or without specific CES1 inhibitor were evaluated by qRT-PCR, seahorse, WB and viability assay.

RESULTS

We found that elevated CES1 expression correlates with worse prognosis in OC patients. Accordingly, we showed that CES1 was basally expressed only in the cell lines EFO21 and OV-56 established from metastatic tumours. Although OVCA-429 and ES2 cell lines established from primary tumours were lacking basal CES1 expression, all OC lines expressed CES1 when cultured under energy stress, suggesting that CES1 could be relevant for their adaptation to harsh metabolic environment. Indeed, pharmacological CES1 blockade by commercially available GR-148672X inhibitor impaired bioenergetic parameters and blocked autophagy flux, thus resulting in significant cell death of all the analysed OC lines. Notably, inhibition of CES1 signalling under energy stress conditions was also able to kill carboplatin-resistant OC cells.

CONCLUSIONS

These data underscore the clinical relevance of this enzyme in OC and suggest that CES1 could be a potential druggable target, especially in chemotherapy-refractory subsets.

P167

OLEUROPEIN TREATMENTS MODULATE THE SIGNALINGS OF EPITHELIAL-MESENCHYMAL TRANSITION AND DNA REPAIR IN SEROUS OVARIAN CANCER MODELS.

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BACKGROUND-AIM

Ovarian cancer (OvCa) is characterized by a high mortality rate among gynecological malignancies worldwide. Metastatic progression from the primary tumor initially occurs through epithelial-mesenchymal transition (EMT) and subsequent regression to MET (mesenchymal-epithelial transition) in the abdominal cavity microenvironment. The pathophysiology of metastatic OvCa is not fully understood, alterations of DNA repair and energy metabolism appear to be involved in the OvCa progression. Olea europaea L. extracts can be used as a potential source of phenols and secorrhoids of both antioxidant and antitumor effects, however in serous OvCa there are very few studies on their effects on EMT and DNA repair. This study investigates the effect of Verbascoside (VB) and Oleuropein (OLE), on serous OvCa models.

METHODS

Chemoresistant cells derived from ascitic fluid, SKOV3 and OVCAR3, and primary tumor A2780 line were treated with VB and OLE. Cell viability and metabolic activity were assayed by MTS. Gene and protein expression were evaluated by qRT-PCR and HCC respectively.

RESULTS

OLE induced a significant inhibition (p=0.04) of SKOV3 cell viability and gene expression of N-Cadherin after 100mM treatment. E-cadherin was found to be increased in OVACAR3 and SKOV3 cells, while Vimentin (Vim) and stemness factor ZEB1 were found to be reduced. After treatment with OLE, BRCA1 was found to be repressed in all three cell lines, regardless of BRCA1 mutational status. In A2780 a significant reduction in mRNA levels of BRCA1/BRCA2, and OGG1/MUTYH was observed after treatment with OLE (50 mM) and Vimentin (100mM).

CONCLUSIONS

Cell viability and metabolic activity tested on OvCa suggest a potential beneficial effect of OLE and VB probably related to the modulation of gene expression of EMT and DNA repair. However, further studies will be needed to explore the molecular pathways underlying these effects.

P168

A RNA APPROACH TARGETING NOTCH2 IN MULTIPLE MYELOMA MAY HAMPER THE PRO-TUMORIGENIC SIGNALS SENT THROUGH EXTRACELLULAR VESICLES

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BACKGROUND-AIM

The communication between multiple myeloma (MM) and the bone marrow (BM) is key for tumor progression and, among the others, it is mediated by Notch pathway. Extracellular vesicles (EVs) are cell-derived nanoparticles surrounded by a lipid bilayer, carrying molecular messengers such as proteins and miRNAs. MM-derived EVs (MM-EVs) mediate the communication with the BM niche. We showed that Notch2 modulation in MM cells reduces MM-EV pro-tumor activity in the BM. This prompted us to assess if Notch2 in MM cells may influence MM-EV cargo.

METHODS

The MM cell line OPM2 was forced to express a shRNA for Notch2 (N2-KD), or the scrambled control by using a lentiviral vector (pTRIPZ). Differentially regulated proteins and miRNAs carried by EVs shed by these cell lines were analyzed through OMIC approaches. Protein variations were investigated by nanoLiquid Chromatography- High Resolution Mass Spectrometry and significantly modulated pathways were identified by Ingenuity Pathways Analysis. Variations in miRNA expression profiles were assessed by TaqMan Low Density Array (Life Technologies).

RESULTS

We found that Notch pathway regulates protein and miRNA content in MM-EVs. The protein analysis performed on MM cells and EVs indicated that, among Notch regulated proteins (90 and 101 up- and down-regulated in MM cells, respectively and 27 and 47, up- and down-regulated in MM-EVs), only two upregulated proteins (Rap-1b and Valine-tRNA ligase 1) and two downregulated ones (Hsp27 and IGLL5) were released from the cells to the EVs, demonstrating the selectivity of EV protein loading. The modulated pathways associated to N2-KD include EIF2 signaling (neg. score), Epithelial Adherents Junction and Ephrin Receptor signaling (pos. score). Among the validated EV miRNA modulated by Notch2, we found mir-331-3P (RQ=0.482; p=0.004), miR-216b-5P (RQ=0.469; p=0.014) and miR-505 (RQ=0.115; p=0.009), previously associated with drug resistance, metastasis and alteration of osteogenesis.

CONCLUSIONS

Here we demonstrated that in MM the aberrantly expressed Notch2 may regulates MM-EV content consistently with its ability to regulate the communication of MM with the BM niche. This finding suggests that a RNA approach targeting Notch2 may hamper the pro-tumor signals of MM-EVs.

P169

DISSECTING THE PECULIAR RIZ2 ROLE IN COLORECTAL CANCER

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BACKGROUND-AIM

Colorectal cancer (CRC) is the third most deadly and fourth most diagnosed cancer worldwide. Despite the progress in early diagnosis and advanced therapeutic options, CRC shows a poor prognosis with a 5-year survival rate of ~45%. PRDM2/RIZ, a member of Positive Regulatory Domain (PRDM) gene family, expresses two main molecular variants, the PR-plus isoform (RIZ1) and the PR-minus (RIZ2). The imbalance in their expression levels in favour of RIZ2 is observed in many cancer types. The full length RIZ1 has been extensively investigated in several cancers where it acts as a tumour suppressor, whereas few studies have explored the oncogenic properties of RIZ2. PRDM2 is often target of frameshift mutations and aberrant DNA methylation in CRC. Accordingly, our analysis of Exome- and transcriptome public datasets available at The Cancer Genome Atlas (TCGA) portal revealed that PRDM2 gene is frequently mutated and transcriptionally deregulated in CRC. However, little is known about its role in CRC and in the regulation of the involved pivotal pathways.

METHODS

In this study, we first evaluated the expression of the different PRDM2 transcripts by in silico analysis on TCGA CRC datasets. Then, we assayed several CRC cell lines by qRT-PCR analysis for the main PRDM2 transcripts and selected DLD-1 cell line, which showed the lowest RIZ2 levels to assess the PRDM2/RIZ2 role in CRC cell behaviour. Particularly, we analysed the possible oncogenic function of RIZ2 by cell viability, growth, colony and organoid formation assays in DLD-1 cells overexpressing RIZ2 isoform.

RESULTS

Our in-silico analysis on TCGA datasets revealed a highly significant downregulation of RIZ1 in CRC samples whereas a RIZ2 increase was observed in the same samples. Noteworthy, the forced RIZ2 expression increased cell viability, growth, colony and organoid formation thus confirming our previous findings on the HEK293 model.

CONCLUSIONS

Our findings add novel insights on the putative RIZ2 tumor-promoting functions in CRC, although additional attempts are warranted to depict the underlying molecular mechanism of action.

P170

IMMUNE RESPONSE AGAINST SARS-COV-2: A COMPARATIVE ANALYSIS BETWEEN VACCINATED SUBJECTS AND SUBJECTS RECOVERED AFTER INFECTION

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BACKGROUND-AIM

A comparison of the reactivity of T cells, in vaccinated subjects and in subjects recovered from the infection, with different serum levels of anti SARS-CoV-2 IgG can shed light on the concept of immune memory and on the state of protection from infection or reinfection from SARS-CoV-2. For this reason, 24 people were enrolled, half of whom were infected with SARS-CoV-2 and the other half were vaccinated with at least two doses of the SARS-CoV-2 mRNA vaccine. In the vaccinated group(never infected), 6 healthy people with an high anti-SARS-CoV-2 IGg antibody titer>1000 BAU/ml and 6 vaccinated subjects but in conditions of immunosuppression were enrolled. In the unvaccinated/cured group, 6 subjects with an anti-SARS-CoV-2 IGg antibody titer> 300 BAU/ml and 6 subjects with a titer <100 BAU/ml were enrolled.A new serological analysis of IgG and a stimulation on whole blood with viral antigens were made.

METHODS

The amount of anti-SARS-CoV-2 anti RBD Spike Protein antibodies was measured using the Maglumi 2000 Snibe®, a chemiluminescence analytical system.Covi-FERON ELISA (Manufactured by SD Biosensor®)uses specialized blood collection tubes, which consist of three Antigen tubes(Original SP, Variant SP and NP Antigen) that use a combination of protein antigens specific to SARS-CoV-2 to stimulate lymphocytes involved in T cell-mediated immunity. Following 37C° incubation period,the amount of IFN-γ (IU/ml) measured by ELISA .

RESULTS

The sample population has an average age of 52 years, 58% of women and 42% of men. Only 33% of the subjects cured and with a low serum titer of anti SARS-CoV-2 IgG antibodies showed a T-cell reactivity, while 67% of the subjects vaccinated and with an antibody titer> 1000 BAU/mL showed T-Cell reactivity to SARS-CoV-2 antigens. In the other two groups the % IFN-γ reactivity was 50%. There was no statistically significant difference in T-Cell reactivity between female and male sex, nor correlation with age. Neither between the two main groups nor between the subgroups was there a statistically significant difference in T-Cell reactivity. However, we found a statistically significant dependence ratio (p <0.001) between the mean values of the serum antibody titer and the concentration of IFN-γ.

CONCLUSIONS

Surely the low sample size is the main limitation of the study. However, an interesting fact can be obtained, namely that the quantitative measurement of serum IgG antibodies to RBD would seem to be representative of cell-mediated immunity against SARS-CoV-2.

P171

EFFECTS OF TREATMENT WITH PPAR-α RECEPTOR MODULATORS ON PRECLINICAL MODELS OF HCMV INFECTION

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BACKGROUND-AIM

HCMV is a Herpesviridae that infects 60-80# of the population. The infection causes severe clinical manifestations in immunocompromised subjects.
Antiviral therapies are limited by dose-dependent toxicity and the acquisition of resistance, while vaccines are not yet available.
Therapies that interfere with cellular metabolism to disfavour viral replication are being studied. In fact, it has been observed that viral replication is related to the alteration of the metabolism of fatty acids in order to favor the biosynthesis of sterols and phosphoinositides for the production of viral structures.
Research on the involvement of PPARs in the dynamics of viral infection is of considerable interest. The studies carried out are limited to PPAR-# and there are no data regarding the correlation between HCMV and the two receptor isoforms α and β / δ.
The purpose of the study was to investigate whether PPAR-α was involved in the dynamics of the viral infection, as it is responsible for the β-oxidation of fatty acids. To this end, it was aimed at verifying whether the modulation of the PPAR-α receptor could interfere with HCMV infection.

METHODS

The study was carried out by treating the HFF-1 and PTJ64i cell lines with a PPAR-α agonist (WY14643) and an antagonist (GW6471). Then, cells were infected with the VR1814 HCMV strain and the effect of the treatments was evaluated by performing analyses of immunofluorescence, expression of viral and cellular transcripts and quantization by qPCR of viral genomes. Furthermore, proteomics was carried out to investigate the effect of HCMV infection on global cellular protein expression.

RESULTS

The morphological analyses showed that treatment with the antagonist GW6471 increases the cytopathic effect. In addition, the immunofluorescence and qPCR analyses respectively showed an increase of viral proteins and genomes in the cells treated with the antagonist GW6471.

CONCLUSIONS

The evidence emerging from this study suggests that inhibition of PPAR-α may be a necessary condition for promoting viral replication. On the other hand, its activation proved to be irrelevant in disfavor the infection. This indicates that the development of antiviral therapies that target PPARs could be important but require further research.

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