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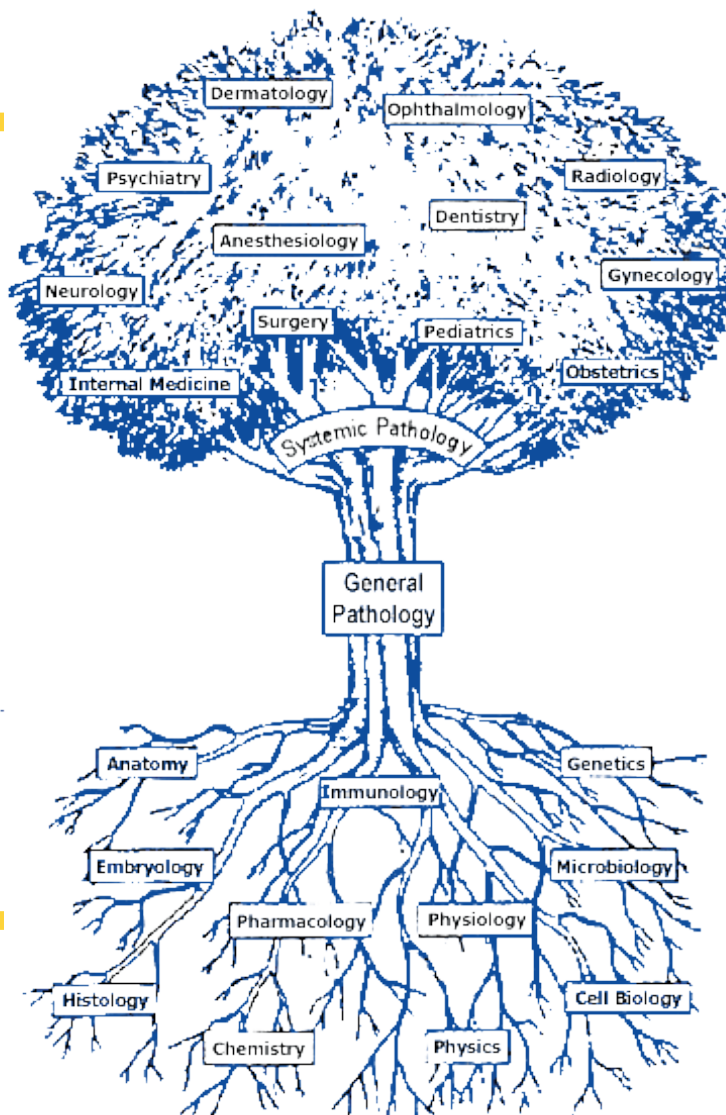
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ABSTRACT BOOK



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P001

INSULIN RESISTANCE RISK CAN PROMOTE IMPAIRED PHOSPHOLIPID TURNOVER VIA SREBFS GENE PATHWAY TRIGGERING TO METAINFLAMMATION IN OVERWEIGHT CARDIOVASCULAR POPULATION

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BACKGROUND-AIM

Phospholipids (PS) are the lipid family mostly represented by phosphatidylcholines (PC) and phosphatidylethanolamines (PE) which are the natural constituent of cellular membranes. Different studies shown the relevance of PS in cardiovascular pathology suggesting them as potential biomarkers for both acute (PC) or chronic (PE) coronary artery events, as well as for clinical and subclinical atherosclerotic diseases. The master regulators of de novo lipid synthesis in mammalian cells are SREBFs genes which are primarily regulated by insulin but also by bioactive metabolites like PS. The aim of this study is to elucidate the SREBs signaling in epicardial (EAT) and subcutaneous (SAT) adipose tissues from CVDs patients with insulin resistance (IR) risk to investigate the potential role of meta-inflammation in impaired PS content via SREBF gene pathways.

METHODS

40 CVDs patients from IRCCS Policlinico San Donato were enrolled and their epicardial adipose tissue (EAT), subcutaneous adipose tissue (SAT) and plasma were drawn during surgery. LV geometry was evaluated by echocardiography and the clinical data were collected. Phospholipids metabolites were quantified by ESI-MS/MS using Quattro Ultima triple-quadrupole mass spectrometer methods. Genomic analysis on SREBs signalling were made using microarray. All the data were analysed using R version 3.3.1.

RESULTS

top 10 differential expression of phospholipid metabolites as PC and PE species in EAT and SAT are observed subdividing CVDs patient according to HOMA and ADIPO Q indexes ($p < 0.05$). Microarray results demonstrated that CVDs patients with IR risk presented a genomic profile that reduced SREBFs gene and up-regulate colin synthesis, the molecular precursor of PS. Interesting PE level in EAT and SAT inhibit SREBFs pathway and activated meta-inflammation through nod-like receptors up-regulation.

CONCLUSIONS

our data suggest that IR risk promotes perturbation in SREBFs expression in EAT and SAT with consequent PS impairment. This deregulation seems to activate nod-like receptors (NLRs) genes in EAT and SAT accompanying meta-inflammation in CVDs patients with IR risk.

P002

LOSS OF DDB2-PCNA PROTEIN INTERACTION DETERMINES THE ACQUISITION OF CANCER CELL PHENOTYPE AFTER DNA DAMAGE INDUCED BY UV-C IRRADIATION

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BACKGROUND-AIM

DDB2 is a protein playing an essential role in the lesion recognition step of the global genome nucleotide excision repair (GG-NER) process; recently, it has been suggested that DDB2 protein is involved in tumorigenesis process although this role is still debated.

We have previously demonstrated that the interaction between DDB2 and PCNA is essential for the proteolytic degradation of DDB2, allowing the correct execution of GG-NER. Using HEK293 cells that stably express the wild-type form of DDB2 (DDB2^{Wt}) or the mutant form unable to bind to PCNA protein (DDB2^{PCNA-}), we have observed that the presence of DDB2^{PCNA-} is associated with a reduced DNA repair efficiency, an increased cell proliferation and ability to invade the surrounding matrix.

METHODS

To select clones from HEK 293 DDB2^{PCNA-} cells resistant to irradiation (UVC 10 J/m²), a clonogenic soft agar assay was performed with non-irradiated and irradiated HEK293 cells, both un-transfected cells and expressing exogenous DDB2. To evaluate protein expression levels, Western blot and flow cytometry were used. To monitor adhesion ability, the iCELLigence biosensor technology was applied.

RESULTS

After irradiation, only the cells expressing the mutated DDB2^{PCNA-} protein were able to form colonies and two clones DDB2^{PCNA-}.1 and DDB2^{PCNA-}.2 (1 and 2) were isolated and expanded. To investigate the capacity of proliferation and resistance to genotoxic agents, these clones were grown in the presence of cell cycle inhibitors, such as caffeine and hydroxyurea, or the anticancer drugs cisplatin and MNNG, respectively. The results demonstrated that DDB2^{PCNA-} cells are significantly more sensitive to the all compounds, but the clones 1 and 2 show a different susceptibility to each treatment. In addition, the two UVC-resistant clones exhibited a reduced capability to adhere to the microelectrodes on the well bottom glass, respect to DDB2^{PCNA-} cell line. Moreover, both these clones expressed a higher amount of SOX2, OCT4 and CD44 proteins, markers of stemness in many human cancer cells.

CONCLUSIONS

These data not only confirm a tumor-like phenotype in DDB2^{PCNA-} cell line, but also show that both UVC-selected clones exhibit increased invasive abilities and a more aggressive behavior than that of DDB2^{PCNA-} cell line.

P003

CDK4/6 INHIBITORS AS A NEW THERAPEUTIC APPROACH IN MESOTHELIOMA MODELS

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BACKGROUND-AIM

Malignant pleural mesothelioma (MPM) is an aggressive disease affecting the pleura and associated with asbestos exposure. Therapeutic actions in the first-line setting are limited to chemotherapy; recently AIFA approved immunotherapy as first-line treatment in unresectable MPM with non-epithelioid histotype.

Loss of the CDKN2A gene is the most common alteration in MPM, thus representing a novel target approach for this untreatable tumor.

The aim of our study was to evaluate the antitumor potential of combining the standard chemotherapy regimen used for unresectable MPM with CDK4/6 inhibitors. Moreover, we evaluated the efficacy of Myrtlecielib, a new compound with promising antitumor activity that targets both CDK4/6 and CDK9 to a similar extent.

METHODS

Experiments were performed in MPM cell lines and in cells derived from pleural effusion of MPM patients. Cell proliferation, cell death, colony formation, cell senescence have been evaluated. In vivo experiments have been performed in an orthotopic rat model of MPM. Myrtlecielib has been developed by the Italian company ViroStatics.

RESULTS

The simultaneous treatment of abemaciclib combined with cisplatin and pemetrexed produced a greater antiproliferative effect than chemotherapy alone in MPM cell lines. This antiproliferative effect was associated with cellular senescence or autophagic cell death, depending on cell type. Notably, the effect of such combination was irreversible and no resumption in tumor cell proliferation was observed after drug withdrawal, suggesting that the addition of abemaciclib could prevent or slow down the acquisition of chemoresistance.

Compared to commercially available CDK4/6 inhibitors (abemaciclib, palbociclib, ribociclib), myrtlecielib exhibited superior antiproliferative efficacy and the ability to induce apoptosis. Efficacy of myrtlecielib has been confirmed also in an orthotopic rat recurrence model of MPM: the treatment with myrtlecielib strongly prevented tumor relapse compared to control.

CONCLUSIONS

Our results proved the therapeutic potential of CDK inhibitors for the treatment of MPM, alone or combined with chemotherapy, and are consistent with the findings of the phase 2 clinical trial Mist2, that show the clinical activity of abemaciclib treatment in MPM patients.

P004

OVERCOMING THERAPEUTIC RESISTANCE IN MUTANT COLORECTAL CANCER BY TARGETING HDAC2-MEDIATED IMMUNE REGULATION

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BACKGROUND-AIM

A large body of clinical and experimental evidence indicates that colorectal cancer is one of the most common multifactorial diseases. Gene expression levels of the epigenetic regulator histone deacetylase 2 (HDAC2) are deregulated in colorectal cancer, and this deregulation is tightly associated with immune dysfunction. Given that the deregulation of these mechanisms promotes cancer progression by altering the balance of genes controlling cell proliferation and death, the objective of this study was to identify a genetic/epigenetic/immunological colorectal cancer signature through a preliminary *in silico* analysis aimed at identifying the pathogenic causes of colorectal cancer associated with expression levels of HDAC2 and two immune system regulators, class II major histocompatibility complex transactivator (CIITA) and beta-2 microglobulin (B2M), in a cohort of patients harboring a common dysregulation of these genes. We next extended the study by investigating a tissue microarray cohort of colorectal cancer patients from a diagnostic/prognostic perspective.

METHODS

Bioinformatic databases, Tissue Microarray Construction, Immunohistochemistry Analysis

RESULTS

We identified patients who presented simultaneous alterations in HDAC2, CIITA, and B2M genes based on mutation levels, structural variants, and RNA expression levels. We found that B2M plays an important role in these alterations and that mutations in this gene are potentially oncogenic. The dysregulated mRNA expression levels of HDAC2 were reported in about 5% of the profiled patients, while other specific alterations were described for CIITA. By analyzing immune infiltrates, we then identified correlations among these three genes in colorectal cancer patients and differential infiltration levels of genetic variants, suggesting that HDAC2 may have an indirect immune-related role in specific subgroups of immune infiltrates.

CONCLUSIONS

Although it is challenging to identify a direct cancer-driving criterion, many hurdles have already been overcome thanks to the combined use of ever-advancing technologies, which are able to translate research from the bench to the bedside, and multi-omics big data that are available on public platforms. Further investigations into the role of HDAC2 deregulation in CRC could provide more conclusive evidence and pave the way toward the use of HDAC2-targeted therapies in the treatment of the disease.

P005

SALL4 IS A CRL3^{REN} SUBSTRATE THAT DRIVES SONIC HEDGEHOG-DEPENDENT MEDULLOBLASTOMA

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BACKGROUND-AIM

Medulloblastoma (MB) is the most common malignant pediatric brain tumor that arises from alterations in cerebellum development. The Sonic Hedgehog variant (SHH-MB) is the best genetically understood, characterized by mutations in the main components of SHH signalling and cytogenetic alterations. We previously identified the Cullin 3 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 the well-known SHH activator HDAC1. However, the mechanisms regulating this event need further investigation.

METHODS

Affinity purification coupled to mass spectrometry was performed to identify REN interactors. Proteomic data were confirmed by co-immunoprecipitation and ubiquitylation assays. Proliferation was evaluated in vitro in primary murine SHH-MB cells and human avatars by IncuCyte® Live-Cell technology. In vivo data were assessed in preclinical models of SHH-MB.

RESULTS

We found that the transcriptional factor and stemness regulator SALL4 is re-expressed in mouse SHH-MB models and its high levels correlate with worse overall survival in SHH-MB patients. We demonstrate that SALL4 is a substrate of CRL3^{REN}, which induces SALL4 polyubiquitylation and degradation. Interestingly, SALL4 binds GLI1 and cooperates with HDAC1 to potentiate the deacetylation and activation of GLI1. Notably, knockdown or pharmacological inhibition of SALL4 by thalidomide suppresses SHH-MB growth both in murine and PDX models.

CONCLUSIONS

Our findings unveil SALL4 as regulator of SHH signaling and highlight the relevance of SALL4 as promising therapeutic target for innovative and more effective interventions for the treatment of SHH-MB.

P006

BLOCKING THE HEDGEHOG-DEPENDENT TUMOR GROWTH BY A NEW SELECTIVE ENDOPLASMIC RETICULUM AMINOPEPTIDASE 1 INHIBITOR

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BACKGROUND-AIM

Aberrant Hedgehog (Hh) signaling occurs in a wide range of human cancers, such as medulloblastoma (MB), the most common brain malignancy in childhood. Recently, we identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1), a key player of the immune response, as a previously unknown positive regulator of the Hh pathway and promising therapeutic target for Hh-driven tumors. However, the lack of availability for highly specific chemical inhibitors for ERAP1 has constrained the progress in this area.

METHODS

To identify novel selective and effective ERAP1 inhibitors, we performed a docking-based virtual screening of a library of natural compounds against crystallographic structure of the ERAP1 catalytic domain and among eleven selected molecules, we identified compound N1 as a potent ERAP1 inhibitor.

RESULTS

We demonstrate that this compound blocks ERAP1 activity and: i) significantly reduces stability of GLI1, thus counteracting Hh signaling; ii) impairs self-renewal ability and clonogenicity of tumor-derived MB stem-like cells; iii) suppresses MB growth in vitro and in vivo.

CONCLUSIONS

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

P007

QUERCETIN TRIGGERS SENOLYSIS OF PRO-TUMOR DOXORUBICIN-INDUCED SENESCENT FIBROBLASTS BY TARGETING AUTOPHAGY

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BACKGROUND-AIM

Cellular senescence, one of the biological processes that drive aging and age-related diseases, is the target of several therapeutic strategies. Senolytics, which selectively eliminate senescent cells, are a highly promising approach, but data on their effects are conflicting, and the mechanisms underlying their action have not been fully elucidated. In this study, we used quercetin as a senolytic agent on doxorubicin (Doxo)-induced senescent fibroblasts, highlighting a new potential mechanism through which it killed senescent cells. Moreover, we analysed the effects of senescent fibroblast conditioned media (CM) on osteosarcoma cells.

METHODS

WI-38 fibroblasts were induced to senescence with Doxo and then treated with quercetin 40 μ M for 3 days. To assess the reduction of senescent cells, we evaluated cell proliferation, cell cycle arrest, senescence-associated β -galactosidase activity (SA- β -gal), senescence-associated heterochromatin foci (SAHF), and cell death by Annexin/7AAD flow cytometry assay. The expression of autophagy markers was analysed by Western Blot and immunofluorescence. Moreover, we investigated colony formation capacity and invasiveness of U2OS treated with fibroblasts CM.

RESULTS

Our results showed that quercetin selectively cleared ~30% of senescent fibroblasts without affecting the proliferating cells. Moreover, our data demonstrate that Doxo-induced senescent fibroblasts, which produce several SASP factors, exhibit increased autophagy. The treatment with quercetin reduced autophagy, which in turn induced an increase in ER proteotoxic stress, demonstrated by the presence of XBP1 spliced mRNA, a marker of unfolded protein response (UPR), resulting in senescent fibroblast death. Moreover, growth and invasion assays demonstrated that the partial clearance of senescent fibroblasts achieved by quercetin treatment was sufficient to curb the pro-tumor effects of their conditioned medium on osteosarcoma cells.

CONCLUSIONS

Our data proves the efficacy of quercetin on Doxo-induced senescent fibroblasts, highlighting an undescribed mechanism of action of the flavonol and demonstrating that the partial clearance of senescent cells could be a promising strategy against chemotoxicity and metastatic progression.

P008

EXPLORING THE HEPATIC INSULIN SENSITIVITY AND INFLAMMATORY RESPONSE VIA NATURAL PRODUCTS: A SPOTLIGHT ON PHOSPHOELEGANIN DERIVATIVES

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BACKGROUND-AIM

Marine natural products are a great source for potential antidiabetic drugs. We have previously shown that phosphoeleganin (PE), a polyketide isolated from the Mediterranean sea squirt *Sidnyum elegans*, and its derived compound PE/3, inhibit protein tyrosine phosphatase 1B (PTP1B) improving insulin signaling in skeletal muscle cells. In contrast, another PE-derived molecule lacking phosphate group, PE/2, does not affect skeletal muscle insulin pathway. In this study, we have evaluated the role of PE, PE/2, and PE/3 on insulin sensitivity in human hepatocellular carcinoma (HepG2) cells.

METHODS

HepG2 cells were treated with phosphoeleganin PE, PE/2 and PE/3 in presence or absence of insulin. Proteins and mRNA levels were analyzed by Western blot and Real-Time RT-PCR, respectively.

RESULTS

Insulin alone stimulates the phosphorylation of its receptor (IR) and of Akt/PKB by 4.9- and 4-fold, respectively. The presence of PE and PE/2 improves the effect of insulin, increasing IR phosphorylation by 10 and 8-fold and Akt/PKB phosphorylation by 8.1 and 6.6-fold, respectively. In contrast PE/3 does not influence the action of insulin in HepG2 cells. Interestingly, PE/2 has an additive effect on insulin-mediated reduction of phosphoenolpyruvate carboxykinase expression. Finally, PE and PE/2 decrease the expression of IL6, known to inhibit the insulin signaling, by 50%.

CONCLUSIONS

The obtained data suggest that the phosphoeleganin-derived compound PE/2 improves hepatic insulin signaling, presumably through the downregulation of IL6.

P009

BOOSTING PERIPHERAL NERVE REGENERATION IN ALS BY THE CXCL12-CXCR4 AXIS

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BACKGROUND-AIM

The peripheral nervous system can regenerate upon injury. Regeneration relies on the intrinsic ability of motor neurons and the contribution of various cell types in the milieu. Nerve terminal regeneration at the neuromuscular junction (NMJ), the synapse controlling movement, involves a signaling axis composed of the chemokine CXCL12 α , released by Schwann cells, and its neuronal receptor CXCR4. Nerve injury triggers CXCR4 expression, while its inhibition delays the process of nerve repair. In Amyotrophic Lateral Sclerosis (ALS), a severe neurodegenerative condition where initial impairments arise at the NMJ before symptom manifestation, the NMJ undergoes cycles of denervation and re-innervation till degeneration overcomes the regenerative capability of the system. We hypothesize that in ALS the regenerative competence can be restored by stimulating CXCR4 using an agonist molecule.

METHODS

To investigate this hypothesis, we employed the SOD1G93A ALS mouse model, imaging technologies, electrophysiological recordings and behavioural and survival tests.

RESULTS

We observed the expression of CXCR4 in the axon terminal of the NMJ during both the pre-symptomatic phase and disease progression. Electrophysiological recordings and behavioral tests showed that chronic administration of NUCC-390, which stimulates the CXCR4-CXCL12 axis, improves motor performance and prolongs survival, thereby delaying disease progression. Furthermore, employing a novel approach developed in our laboratory, we discovered that NUCC-390 preserves respiratory function in SOD1G93A mice. Currently I'm testing if this axis is involved in peripheral neurorepair in human samples. I am conducting experiments on biopsies obtained from ALS patients and additionally I'm using MNs derived from patients' iPSCs to test the translational potential of boosting repair pathways to counteract degeneration in ALS.

CONCLUSIONS

The present research holds significant translational value. On one hand, it provides experimental evidence that supporting nerve regeneration may be a new exploitable strategy to improve therapeutic protocols and regenerative outcomes in ALS. On the other hand, it identifies NUCC-390 as a potential candidate molecule to counteract ALS as well as other neurodegenerative diseases.

HYPERACTIVE NOTCH3 DERAILED CXCR4-DEPENDENT MATURATION OF DN THYMOCYTES TO SUSTAIN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PROGRESSION.

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BACKGROUND-AIM

T-cell acute lymphoblastic leukemia (T-ALL), an aggressive lymphoproliferative disorder results from the malignant transformation of T-cell progenitors and originates in the thymus. Notch1 activating mutations or Notch3 overexpression are frequently found in T-ALL patients. In the thymus, T-cell precursors progressively mature from bone marrow (BM)-derived CD4-CD8- (DN) to CD4+CD8+ (DP), to mature single CD4+ or CD8+ T-cells. Notch, CXCR4, and pre-TCR cooperatively drive this developmental program, and regulate the survival and proliferation to progress maturation of thymocytes. Overexpression of an active intracellular form of Notch3 receptor in a mouse model (N3-ICtg) deregulates early immature thymocyte maturation and drives the development of an aggressive T-ALL form, characterized by impaired T-cell maturation and immature DP cells in blood circulation. We study the role of CXCR4 in deregulated molecular and functional mechanisms driving T-ALL progression in our N3-ICtg model by microRNAs.

METHODS

FACS analysis of DN T cells in WT and N3-ICtg mice assess CXCR4 expression in combination with T-cell differentiation markers (CD3 ϵ , CD5). We evaluate the proliferation and apoptosis of the DNCD3 ϵ + T subset as a function of CXCR4 expression. Based on Bioinformatic studies of the 3'UTR of the *cxcr4* gene, we detect the expression of Notch3-induced miRNAs on sorted DNCD3 ϵ +CXCR4+ and DNCD3 ϵ +CXCR4- cells. We transfect an N3-ICtg-derived cell line, N3-232-T, with mimic-139-5p and analyze CXCR4 expression by qRT-PCR and FACS analysis.

RESULTS

In our Notch3-induced T-ALL model, we investigate the aberrant maturation of the DN T-cells along tumor progression. In the transgenic thymus, the CXCR4 cell-surface expression is low or even absent on DN CD3 ϵ + T-cells, which in turn appear to be overrepresented in N3-ICtg as compared to WT mice. This suggests that hyperactive Notch3 negatively regulates CXCR4 in DNCD3 ϵ + cells, interfering with their migration ability and DN-DP transition. The inverse correlation between CXCR4 expression and Notch3-induced miRNAs may suggest a miRNA-mediated mechanism.

CONCLUSIONS

Our results suggest that Notch3 alters the microRNA expression profile and down-regulates the CXCR4 receptor to select an aberrant thymocyte subsets, that sustain leukemia progression. Our present findings may be a more in-depth understanding of the mechanism underlying T-ALL pathogenesis and can propose future perspectives for the development of new therapeutic approaches.

P011

OSIMERTINIB-RESISTANT NSCLC CELL LINES SURVIVE UNDER LOW GLUCOSE ENVIRONMENT DEPENDING ON OXIDATIVE PHOSPHORYLATION

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BACKGROUND-AIM

The mutant-selective EGFR tyrosine kinase, osimertinib (OSI), has been approved for first-line treatment in NSCLC patients. Despite its efficacy, patients inevitably develop acquired resistance. Metabolic rewiring in cancer cells is crucial for maintaining energy levels, producing molecules necessary for cell division, redox balance, and playing an important role in drug resistance and stress conditions.

METHODS

Studies were conducted on a panel of NSCLC cell lines using radiolabeled analogues and the Seahorse XFp analyzer to analyze metabolic profiles, as well as spectrophotometry, fluorescence microscopy, and flow cytometry for proliferation/viability assays.

RESULTS

Unlike OSI-sensitive cells, resistant cells can survive under low-nutrient conditions. The low glucose environment induced activation of the energy sensor adenosine monophosphate-dependent protein kinase (AMPK), which stimulated different energy stress adaptive responses such as increased glucose uptake and mitochondrial metabolism. OSI-resistant cells exhibited increased uptake and consumption of glucose and glutamine at low glucose environment and a heightened dependency on mitochondrial metabolism, leading to a shift towards oxidative phosphorylation (OXPHOS). Moreover, pharmacological inhibition of glutamine transport and consumption resulted in the inhibition of cell proliferation and survival of OSI-resistant cells by reducing mitochondrial respiration and energy production. Additionally, at low glucose, resistant cells displayed an increased dependency on pyruvate for mitochondrial respiration, as shown by decreased mitochondrial oxygen consumption upon inhibition of pyruvate transport into mitochondria. We also demonstrated that the inhibition of OXPHOS hindered cell proliferation at low glucose. Taken together, all these results reflect the increased dependence on mitochondrial metabolism at low glucose levels.

CONCLUSIONS

OSI-resistant NSCLC cell lines have adapted to survive in a low-nutrient environment and under energy stress by increasing glucose uptake and shifting towards mitochondrial metabolism and dependence on OXPHOS when grown at low glucose levels. Inhibition of mitochondrial glutamine oxidation and OXPHOS reduces the viability of OSI-resistant cells.

P012

BROMODOMAIN-CONTAINING PROTEIN BRPF1 AS NEW POTENTIAL THERAPEUTIC TARGET FOR ENDOCRINE THERAPY RESISTANCE BREAST CANCERS

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BACKGROUND-AIM

Approximately 70% of Breast Cancer (BC) are hormone-responsive and express Estrogen Receptor alpha (ER α) and endocrine therapies (ET) represent the mainstay treatment for patients affected by this BC subtype. Resistance to these therapies is a major challenge in clinical management of these diseases, hence new therapeutic approaches against ET-resistant tumors are needed and actively sought after.

METHODS

Computational analysis of genome-wide 'drop-out' screenings combined with ER α interactome profiling revealed a set of 40 genes, including the bromodomain BRPF1. The involvement of BRPF1 in mediating ER α actions on genome and evaluation of functional impact in antiestrogen-sensitive and -resistant BC cell models was investigated by Chromatin Immunoprecipitation and sequencing (ChIP-Seq), siRNA-mediated knock-down or pharmacological inhibition of BRPF1, followed by steady-state transcriptome profiling (RNA-seq). Cellular and functional assay were also performed to evaluate the functional impact interplay of BRPF1-ER α in antiestrogen-sensitive and -resistant BC cell and in Patient Derived Organoids (PDOs).

RESULTS

BRPF1 acts as an epigenetic 'reader' that recognizes specific histone marks to vehiculate the histone acetyltransferase activity of the MOZ/MORF complex to regulatory regions of target genes. Global analysis of ER α and BRPF1 binding to the genome showed co-recruitment of both proteins on a significant subset of chromatin sites, resulting in regulation of genes involved in cell death and proliferation. Moreover, BRPF1 knock-down or pharmacological inhibition downregulates ER α expression, reduces cell proliferation, activates apoptotic mechanisms and induces transcriptome changes resulting among others, in inhibition of hormone signaling, cell cycle and EMT pathways both in anti estrogen-sensitive and -resistant BC cells and BC PDOs.

CONCLUSIONS

Altogether, these results indicate that BRPF1 is an essential component of the estrogen signaling in BC and its impact on cell proliferation and survival in ET-resistant BC reveals new actionable therapeutic targets for treatment of these aggressive tumors.

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P013

IDENTIFICATION OF ESSENTIAL LNCRNAs ASSOCIATED WITH ESTROGEN RECEPTOR ALPHA AS NOVEL TARGETS FOR HORMONE-RESPONSIVE BREAST CANCER TREATMENT

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BACKGROUND-AIM

In latest years, the discovery of RNA-based therapeutics highlighted a novel way to target either coding or non-coding molecules for targeted treatment of multiple diseases. In breast cancer (BC), a plethora of deregulated non-coding RNA molecules were described as putative drug agents. In the context of antiestrogen resistance occurrence (~30% cases), the RNA binding activity of the estrogen receptor α (ER α) pointed out novel druggable molecules to overcome this phenomenon. Among ER α -associated RNAs, lncRNAs were selected for further investigation due to their key role in nuclear processes such as transcriptional regulation and epigenetic chromatin modifications.

METHODS

Native nuclear RNA immunoprecipitation coupled to next generation sequencing (RIP-Seq) was performed in exponentially growing MCF-7 cells. This allowed the identification of RNA molecules interacting with ER α in nuclear multi-molecular regulatory complexes formation. The functional effect of selected lncRNAs on cell proliferation, migration, apoptosis and estrogenic signaling modulation was evaluated by using antisense-oligonucleotides (ASO) for their silencing.

RESULTS

In this study, among the RNAs molecules associated with ER α in the nuclear compartment, the lncRNAs PVT1, FGD5-AS1 and EPB41L4A-AS1, described to be essential in BC cell models, have been selected for further investigation. Functional assays, through ASO-mediated lncRNA gene silencing, were performed in luminal-like, triple negative and antiestrogen resistant BC cell models, showing significant reduction of proliferation and migration rate and increase of apoptosis. In addition, the involvement of ER α -interacting lncRNAs in the estrogenic signaling was investigated with promising effects.

CONCLUSIONS

The results obtained suggested that ER α -interacting lncRNAs may represent key functional components of the estrogen signaling pathway in luminal-like BC cells and novel potential targets to overcome resistance in endocrine therapies.

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P014

THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS IN TUMOR MICROENVIRONMENT OF NOTCH DEPENDENT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: POSSIBLE INTERACTION WITH NK CELLS THROUGH THE PD-1/PD-L1 AXIS

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BACKGROUND-AIM

Notch signaling is crucial for T cell development and its dysregulation induces 'T-cell Acute Lymphoblastic Leukemia' (T-ALL). Notch3 transgenic mice (N3-tg) represents a consolidated model of T-ALL, in which tumoral T cells drive accumulation of 'Myeloid-Derived Suppressor Cells' (MDSCs). MDSCs are immature cells that inhibit immune responses to create a tumor permissive environment. Programmed cell death 1 (PD-1) is an inhibitory receptor that suppress T-cell activation/proliferation, upon interaction with the PD-L1 ligand. MDSCs help tumor progression through different mechanisms, including the expression of PD-L1 and consequent inhibition of PD-1+ cells in tumor microenvironment, such as T and NK cells. Our aim is to define MDSCs as a possible target of therapy for T-ALL, aimed to reactivate immune responses, with particular regard to that of NKs and possibly, through modulation of the PD-1/PD-L1 axis.

METHODS

We used BMs and spleens from N3-tg mice and wt controls, at different disease stages. We evaluated numbers and percentages of PD-1+ and PD-L1+ cells within NK- and MDSC-cell subsets, by FACS assay. We examined by FACS analysis the activity of NKs, purified by FACS-assisted cell sorting, by co-culturing them with YAC-1 T lymphoma cells as targets, in a standard cytolytic assay.

RESULTS

Our data demonstrated that in N3-tg mice numbers and function of NK cells are reduced, whereas percentages of them that express PD-1 increases, during disease progression. Concomitantly, accumulating MDSCs express PD-L1 at higher percentages than in wt controls and are able to impair NK activity. Finally, preliminary experiments of treatment of N3-tg mice with anti-PD-L1 antibodies inhibits progression of T-ALL, by reducing numbers of tumoral T cells and MDSCs, while expanding NK subset. Finally, preliminary results indicates that treatment of N3-tg mice with anti-PD-L1 antibodies inhibits progression of the disease.

CONCLUSIONS

We suggest that in Notch-dependent T-ALL, MDSCs may hinder the anti-tumor activity of NKs via the PD-1/PD-L1 axis, thus favouring disease progression. Then, molecules and cells of this network may be explored as possible prognostic markers, as well as targets of innovative therapies.

P015

THE MITOCHONDRIAL FISSION EFFECTOR DRP1 IS A NEW THERAPEUTIC VULNERABILITY IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Alterations in mitochondrial dynamics, due to an increase in the expression or activity of the main GTPase effector Drp1, are observed in several disease contexts. Taking advantage of different gain- or loss-of-function strategies, we investigated Drp1 expression pattern and therapeutic potential in plasma cell (PC) dyscrasia models.

METHODS

Proteasome inhibitors (PI)-resistant cell lines were generated by stepwise exposure to increasing concentrations of the drugs. Mitochondrial structure was assessed by TEM. Cell viability was assessed by Cell Titer Glo assay. 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 retrieved by GSE5900, GSE13591 and GSE2658 datasets. Subcutaneous MM xenografts were used for studying in vivo tumorigenicity.

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, and associated with worse overall survival of MM patients under PI-based regimens in the CoMMpass database. MM cells, especially those resistant to PI, displayed upregulation of the main fission effectors, namely Drp1, its active S616 phosphorylated form and the Drp1 receptor MFF. TEM analysis revealed rearrangements suggestive of ongoing mitochondrial fission in primary CD138+ MM cells as well as in MM cell lines, especially in PI-resistant cell lines or in PCs obtained from RRMM patients. Genetic and pharmacological targeting of Drp1, by mDIVI-1 or a novel ellipticine Drp1 inhibitor, reduced MM cell viability, even in co-culture with bone marrow stromal cells, and synergized with PI, while sparing healthy PBMCs. Mechanistically, RNA-seq analysis highlighted apoptosis and ferroptosis as the most significant upregulated gene sets upon Drp1 inhibition, with down-regulation of lipogenesis-related transcription factors, as MYC and SREBF1/2, and reprogramming of lipid metabolism, as indicated by inhibition of cholesterol and triglycerides total levels. In vivo, both genetic and pharmacological Drp1 targeting reduced the growth of MM xenografts while prolonging survival.

CONCLUSIONS

These data shed light on dysregulated mitochondrial dynamics, and pave the way to the use of Drp1 inhibitors as a novel therapeutic option especially in the PI-resistant setting.

P016

A NOVEL INTERPLAY BETWEEN POLYAMINES, EIF5A AND MYC IN COLORECTAL CANCER

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BACKGROUND-AIM

Polyamines are small polycations involved in many cellular functions and their intracellular content is often increased in colorectal cancer, the third-most lethal cancer worldwide. A key role in polyamine biosynthesis is played by ornithine decarboxylase (ODC) and the irreversible ODC inhibitor DFMO was shown to efficiently prevent tumor growth in different settings, leading to clinical trials. However, the therapeutic benefit of this approach was limited, likely because of increase in polyamine uptake. Hence, different strategies targeting polyamine-regulated downstream effectors appear to be better approaches. One of them is eIF5A, a translational regulator activated by spermidine that alleviates ribosome stalling at specific pausing motifs. Recent studies have shown that EIF5A is overexpressed in various cancers, including CRC, where it correlates with poor prognosis.

METHODS

Using pharmacological and genetic inhibition approaches, we have studied the effect of DHPS-eIF5A axis ablation in vitro in CRC cell lines and in vivo in a preclinical model of CRC and FAP. A transcriptomics analysis allowed the identification of EIF5A key downstream targets and the molecular mechanism of action was defined by polysomal fractionation and a CRISPR-Cas9 based approach.

RESULTS

Inhibition of DHPS efficiently suppresses CRC cell growth and intestinal tumorigenesis in mice, by directly inhibiting eIF5A-mediated elongation of MYC at five distinct pausing motifs in MYC coding sequence (CDS). Because MYC regulates ODC expression, these findings highlight a positive feedback loop, whereby MYC regulates the polyamine biosynthetic pathway through ODC expression, while ODC and polyamines promote MYC biosynthesis through the hypusination pathway. Since this feedback loop is likely amplified in colorectal cancer, we have investigated the effect of combined inhibition of both branches of the feedback loop by targeting ODC and DHPS in combination. We have observed that, while inhibition of each individual pathway causes cytostatic effect, the combinatorial approach causes a synergistic response and induces cell death of CRC cells, likely reflecting two distinct modes of suppression of MYC, by preventing translational elongation and initiation.

CONCLUSIONS

Together, our data illustrate a novel strategy for CRC treatment, based on the combined suppression of ODC and eIF5A which hold promise for the treatment of CRC.

P017

NOVEL N-(HETEROCYCLYPHENYL)BENZENESULFONAMIDE SHARING AN UNREPORTED BINDING SITE WITH TCF-4 AT THE β -CATENIN ARMADILLO REPEATS DOMAIN AS ANTICANCER AGENT

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BACKGROUND-AIM

β -catenin promotes the growth of several type of cancers, including colon cancer, hepatocellular carcinoma, pancreatic cancer, lung cancer and ovarian cancer. In the last decades, intensive efforts to discover specific inhibitors of the Wnt/ β -catenin signaling pathway have been documented. Despite intensive efforts, no inhibitors of the Wnt/ β -catenin signaling pathway have been approved for the clinical treatment of cancer. We synthesized novel N-(heterocycliphenyl)benzenesulfonamides as β -catenin inhibitors and tested them on Wnt/ β -catenin-dependent cancer cell lines.

METHODS

We identified potential Wnt/ β -catenin inhibitors through structure-based virtual screening. Cell viability was evaluated by XTT or MTT colorimetric assays. 30 x 10³ cells/well were seeded in 96-well plates and exposed to increasing concentrations of different compounds (range 0-300 nM) for 48 or 72 h. The luciferase report assay of Topflash/Fopflash was used to measure the activity of Wnt/ β -catenin signaling pathway. In co-immunoprecipitation assays 2 x 10⁴/cm² HCT116 cells were transfected with pcDNA/Myc, TCF4 and cotreated with 50 nM LiCl and 50 nM compound for 24 h. In vivo xenograft experiments were performed on 10 week-old female BALB/Cnu/nu mice. 1 x 10⁸ HCT116 cells/mL were inoculated subcutaneously into BALB/Cnu/nu mice, and intraperitoneal injections of 100 μ L compound (25 mg/kg) every 2 days after tumorigenesis were performed.

RESULTS

In crystallographic studies of the β -catenin armadillo repeats domain, compound 9 superimposed to Tcf-4 highlighting a common binding site within the hotspot binding region close to Lys508. In co-immunoprecipitation study in cells transfected with Myc-tagged TCF-4, compound 9 abrogated the association between β -catenin and TCF-4. Compound 9 showed strong inhibition of the luciferase activity, induced in vitro cell death in SW480 and HCT116 cells and inhibited in vivo tumourigenicity of a human colorectal cancer line HCT116.

CONCLUSIONS

We described the synthesis and antitumor activities of novel N-(heterocycliphenyl)benzenesulfonamide β -catenin inhibitors. These findings highlight the potential of this novel class of β -catenin inhibitors as anticancer agents and pave the way for further development.

P018

THE CONTRIBUTION OF THE GLYOXALASE PATHWAY TO INFLAMMATORY PATHOLOGY IN CYSTIC FIBROSIS

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BACKGROUND-AIM

The glyoxalase system, consisting of glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2), is a highly conserved and ubiquitous pathway involved in the removal of the potent pro-inflammatory glycolytic metabolite, the methylglyoxal (MG). MG is the major precursor of advanced glycation end products (AGEs) that, by binding to receptor for AGEs (RAGE), activate pathogenic inflammation. Studies have shown that increased accumulation of AGEs occurs in multiple diseases, including pulmonary diseases. Because a chronic inflammatory state to which up-regulation of RAGE and inhibition of the indoleamine 2,3 dioxygenase (IDO)1 tolerogenic pathway is a characteristic feature of the airways of Cystic fibrosis (CF) patients, a role for the glyoxalase pathway to lung inflammatory morbidity CF is plausible but not demonstrated.

METHODS

We resorted to in vivo and in vitro preclinical models of CF to define the possible contribution of the glyoxalase system to inflammatory pathology in this human disease.

RESULTS

We found that the expression and activity of Glo1 was defective in the lung of Cfr^{F508del} mice upon infection with *Aspergillus fumigatus*, and this contributed to the occurrence of pathogenic inflammation in response to the fungus. Glo1 was also down-regulated in human CFBE41o-cells transduced with p.Phe508del-CFTR exposed to *A. fumigatus*, and in human bronchial cells (HBE) from CF patients homozygous for Δ F508 mutation. Along with the Glo1 down-regulation, up-regulation of Glo2 was also observed, both findings being recapitulated in *Aspergillus*-infected, IDO1-deficient mice, a finding pointing to a possible regulatory network between the Glo1/2 and the IDO1 pathways. Of interest, both Glo1 and IDO1 activities were restored by treatment with the anti-inflammatory agent anakinra.

CONCLUSIONS

These data point to a possible link between the IDO1 and the glyoxalase pathways in CF and its therapeutic exploitation for the development of novel anti-inflammatory therapy in CF.

P019

ACTIVITY OF MTDNA AS A DAMP ON MONOCYTES FROM ELDERLY SUBJECTS

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BACKGROUND-AIM

Mitochondrial components, including mitochondrial DNA (mtDNA), when released extracellularly, can act as “damage-associated molecular pattern” (DAMP) agents and cause inflammation. Elderly people are characterized by a low-grade, chronic inflammation defined “inflamm-aging”. We previously showed that plasma mtDNA levels increase with aging and that subjects with the highest mtDNA plasma levels have the highest amounts of pro-inflammatory cytokines tumour necrosis factor (TNF)- α and interleukin (IL)-6. In vitro stimulation of monocytes with mtDNA concentrations observed in plasma from elderly people resulted in an increased TNF- α production. Nevertheless, it is not known if the capability of mtDNA to activate a pro-inflammatory response is modulated with aging, and if antigen presenting cells from aged people can be activated by mtDNA.

METHODS

We treated isolated monocytes from young and ultra-nonagenarians donors with purified mtDNA concentrations found in plasma, in combination with pro-inflammatory stimuli. We evaluated mtDNA/toll like receptor 9 (TLR9) internalization and colocalization by ddPCR and confocal microscopy, and cytokine release by ELLA.

RESULTS

Monocyte expression of the mtDNA receptor TLR9, showed a slight reduction with aging. mtDNA was internalized only in cells pre-treated with LPS and co-localized with TLR9. When internalization occurred, monocytes released pro-inflammatory cytokines at higher levels than cells treated with LPS alone. Monocytes from old subjects showed a partial impairment in mtDNA uptake, and a lower capability to activate TLR9 downstream pathway. However, the capability to secrete pro-inflammatory cytokines was not compromised, suggesting that compensatory mechanisms allowed mtDNA-driven activation of monocytes in old people. We also investigated if a similar effect could be observed in microglial cells but to no avail, suggesting that mtDNA activation of TLR9 pathway is observed in monocytes is not a general phenomenon.

CONCLUSIONS

mtDNA can activate a pro-inflammatory response in also monocytes from old subjects, suggesting that this mechanism of activation is preserved with aging and can contribute to the onset of inflamm-aging.

FUNCTIONAL ANALYSIS OF MITOCHONDRIA AND THE IMPACT OF OXIDATIVE STRESS IN FIBROBLASTS FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND-AIM

Amyotrophic lateral sclerosis (ALS) is a motoneuron disorder characterized by a progressive deterioration of both upper and lower motor neurons. Approximately 10% of ALS cases follow an autosomal dominant inheritance pattern. Mitochondrial (mt) impairment is a common characteristic of several neurodegenerative diseases, including ALS. Alterations of mt morphology, metabolic activity, oxidative phosphorylation, and production of reactive oxygen species (ROS) has been observed in ALS patients. Thus, we report a functional analysis of mitochondria and the impact of oxidative stress in different study groups ALS patients.

METHODS

We set up a primary culture of fibroblasts from a male patient with pA382T mutation in TARDBP gene and from two monozygotic discordant twins, one diagnosed with ALS and carried A505G mutation in the progranulin gene, while the other twin was healthy. We analyzed TDP-43 and progranulin level by immunoblot, mt morphology by confocal microscopy, mt respiration through Seahorse technology, and ROS production, and MMP by fluorescence assays. We finally quantified cytosolic mtDNA levels in ALS and CTRL fibroblasts.

RESULTS

Fibroblasts from patient carrying pA382T showed a deep alteration of cell proteome. TDP-43 levels were similar to CTRL fibroblasts, but a higher fraction localized in mitochondria. Mitochondrial network was fragmented, and the organelles smaller and more spheric. Basal cell respiration was reduced. MtDNA levels appeared normal, but a higher amount of mtDNA was present in the cytosol. Concerning discordant twins, Progranulin levels were similar in patient and control; fibroblasts from affected twin were morphologically different from and grew at slower than control. Mitochondrial network and morphology showed no differences between CTRL and ALS. Higher basal and maximal respiration and a higher mitochondrial membrane potential were observed in the fibroblasts of the ALS-affected twin.

CONCLUSIONS

This data confirms that mitochondria from ALS patients displays a wide range of functional and morphological alterations, but with a high degree of heterogeneity, indicating that the mechanistic links mt impairment and ALS pathogenesis greatly vary on the basis of the genetic alterations.

CELL FREE MITOCHONDRIAL DNA (MT-DNA) AS A BIOMARKER IN MILD TRAUMATIC BRAIN INJURY (MTBI) IN BOXING

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BACKGROUND-AIM

Concussion, a type of mild traumatic brain injury (mTBI), is an injury encountered in various sports, including boxing and American football. Continuous mTBI could increase circulating free mitochondrial DNA (cf-mtDNA) in both plasma and cerebrospinal fluid, activate the peripheral innate immune system and contribute to systemic inflammation, associated with neurological damage. However, this possible role of mtDNA in sport – and in particular in boxing - has not been investigated yet.

METHODS

Ten male non-professional boxers, aged 18-36 years, were selected to participate in one sparring match per week for three consecutive weeks. Venous blood samples were processed following standard blood separation protocols to avoid platelets contamination (total mtDNA). Plasma firstly was centrifuged at 18,000g for 30 min (mtDNA1); then plasma was subjected to ultracentrifugation at 100,000g for 2 h to obtain naked DNA (mtDNA2). Nuclear DNA and mtDNA were quantified by Droplet Digital PCR (ddPCR). Light-chain neurofilaments (NFL), pro-inflammatory cytokines (IL6, IL8, IL1b, TNFa) and anti-inflammatory cytokines (IL2, IL10, IL4, IL13) were quantified by ELLA.

RESULTS

We observed an increase in mtDNA concentration after a match in total mtDNA, mtDNA1, and mtDNA2 samples. mtDNA levels returned to baseline within the subsequent matches, except for mtDNA1, which progressively increased over the weeks. NFL concentration remained stable after each match. IL-6 plasma concentration increased after each match and then returned to pre-match values. The other pro-inflammatory cytokines showed a similar trend, although not statistically significant. The anti-inflammatory cytokines IL2 and IL10 showed a similar trend although less pronounced, while IL4 and IL13 were below the detection limit.

CONCLUSIONS

Plasmatic mtDNA -regardless the form - increased after a sparring match, while changes in a match series were observed only in naked mtDNA. The potential association between cf-mtDNA and mTBI could suggest the use of cf-mtDNA as a possible marker of neurological damage.

CCRL2 EXPRESSION BY ALVEOLAR CAPILLARY ENDOTHELIAL CELLS CONTROLS NK CELL HOMING IN LUNG CANCER

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BACKGROUND-AIM

The selected expression of chemotactic receptors regulates the homing of leukocytes to tissues. C-C Chemokine Receptor-Like 2 (CCRL2) is a chemokine-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 in several pathophysiological contexts, as extensively demonstrated by our group. Here we reported that the CCRL2/chemerin/CMKLR1 axis represents a mechanism for the homing of NK cells to the lung. CCRL2 constitutive or conditional endothelial cell targeted ablation, or deletion of its ligand chemerin, were found to promote tumor progression in a Kras/p53Flox (TK) lung cancer cell model.

METHODS

8-12 weeks old C57BL/6J mice with the genotypes WT, Ccr12 KO, TK Ccr12 WT and KO, Cxcr3 KO, Cx3cr1 KO, Chemerin KO, Cdh5Cre/ERT2;Ccr12f/f, Cdh5Cre/ERT2; Akr2 f/f and Cmk1r1 KO were investigated by FACS analysis and histology. NKp46+ CD127+ ILCs (Innate Lymphoid Cells) from lung tumors of TK Ccr12 WT and KO were investigated by scRNA-seq. To characterize the expression of CCRL2 by lung ECs, mouse and human scRNA datasets from healthy and lung tumor specimens were queried (E-MTAB-7458, GSE160876, GSE165063). LUAD transcriptomic and epigenetic datasets were retrieved from GDC.

RESULTS

The genetic ablation of the CCRL2/chemerin/CMKLR1 axis was associated with a reduced recruitment of CD27-CD11b+ mature NK cells. Other chemotactic receptors identified in lung infiltrating NK cells by scRNA-seq, such as CXCR3, CX3CR1 and S1PR5 were found dispensable in the regulation of lung NK cell infiltration and tumor growth. scRNA-seq identified CCRL2 as the hallmark of general alveolar lung capillary endothelial cells. CCRL2 expression was epigenetically regulated in lung endothelium, and it was upregulated by the demethylating agent 5-aza 2'-deoxycytidine (5-Aza). In vivo administration of low doses of 5-Aza induced CCRL2 upregulation, increased recruitment of NK cells and reduced lung tumor growth.

CONCLUSIONS

These results identify CCRL2 as a new NK cell lung homing molecule that has the potential to be exploited to promote NK cell-mediated lung immune surveillance.

BMI-1: A NEW TARGET TO FIGHT NEUROBLASTOMA CHEMORESISTANCE BY INDUCING FERROPTOTIC DEATH

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BACKGROUND-AIM

Neuroblastoma (NB) is a pediatric cancer that is characterized by a rich heterogeneity ranging from a spontaneous regression to the high-risk (HR) form. Initially, HR patients respond well to therapy, but about a 15-20% of them acquires chemoresistance. Current therapies counteract NB progression by inducing oxidative stress and it has been demonstrated that the long-term treatment is able to induce chemoresistance by increasing cancer cell antioxidant defense. Thus, it is crucial to investigate the molecular mechanisms underlying chemoresistance in order to identify the potential targets involved in the adaptive response of NB cells to the pro-oxidant therapy. In this context, our previous studies showed that etoposide-resistant NB cells (HTLA-ER) display downregulation of miRNA-15 and -16 and overexpression of their target, BMI-1. BMI-1 is an oncoprotein involved in tumorigenicity and in the synthesis of glutathione (GSH), the most important non-enzymatic antioxidant. Indeed, HTLA-ER cells exhibit high amount of GSH, which maintain low the levels of hydrogen peroxide (H₂O₂) favoring cell survival. Based on these findings, the aim of this study was to investigate the role of BMI-1 as a potential target to fight chemoresistance.

METHODS

Parental HTLA-230 cells were chronically treated with etoposide at clinically used doses, leading to the selection of HTLA-ER cells. Both populations were treated with 35 nM PTC596, that is a BMI-1 inhibitor used in several clinical trials, and were analyzed in terms of cell viability (MTT assay), BMI-1 and epithelial-mesenchymal transition (EMT) protein levels (immunoblotting), clonogenicity and stemness, H₂O₂ production (DCFH-DA assay), GSH levels (HPLC analysis) and lipid peroxidation (BODIPY assay).

RESULTS

72h treatment with PTC596 decreased BMI-1 expression, reduced the viability of both cell populations and the expression of EMT-related proteins, leading to a decrease in NB clonogenicity and stemness. PTC-induced effects were accompanied by GSH depletion, H₂O₂ overproduction and induction of lipid peroxidation, all features of a ferroptotic death.

CONCLUSIONS

These results suggest, for the first time, that PTC596, by inhibiting BMI-1, can induce ferroptosis and could be a promising strategy to fight chemoresistance.

P024

IMMUNE-INFLAMMATORY PARAMETERS IN SEMI AND SUPER-CENTENARIANS

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BACKGROUND-AIM

INFLA-score and the determination of the aging-related immune phenotype (ARIP), which describe the immunoinflammatory status of older people, may represent a suitable tool in the evaluation of biological ageing. The INFLA-score contributes to evaluate the possible synergistic effect of inflammatory biomarkers, that may produce multicollinearity when simultaneously studied, ignoring the variability presented by the differences in units, mean intakes and biological actions.

METHODS

INFLA-score was determined on a population of Sicilians (N= 250, age range 19-111 years), subdivided in adults (19-70 years), older adults (70-90 years), LLIs (90-105 years) and oldest centenarians (>105 years). For INFLA-score calculation, the 10-tiles of CRP, Leukocyte count, Platelet count, Neutrophil/Lymphocyte ratio levels were generated. The four different 10-tiles of biomarkers were scored from lower levels (from -4 to -1) to higher levels (from +4 to +1) giving 0 score to two intermediate values. The sum of the four components gives the INFLA-score, that ranges between -16 and 16. For ARIP indicators, based on age-related changes in T cell distribution, a sample of 54 Sicilians, aged between 19 and 110 years (adults n=20, older adults n=15; LLIs n=11; oldest centenarians n=8) was considered. We determined: TN (naïve)/(TCM (Central Memory)+TEM (Effector Memory)+TEFF (Effector)) (referred as TN/TM) in CD4+ and CD8+ T cells, previously associated with biological and chronological ageing as well as with age-related chronic conditions.

RESULTS

As expected, the values of INFLA-score significantly increased with age. However, dividing the subject into groups (adults, older adults, LLIs and oldest centenarians), only LLIs showed a higher INFLA-score than adults. Concerning ARIP, the values of TN/TM CD8+, but not those of TN/TM CD4+, significantly decreased with age. While dividing the subject into the same age groups, only older adults and LLIs showed lower TN/TM CD8+ values than adults.

CONCLUSIONS

The data regarding INFLA-score and ARIP showed the peculiarity of the oldest centenarians in terms of inflammatory and immune status, providing further evidence to support the idea that controlling immune-inflammatory responses plays a key role in achieving extreme longevity.

EPHA2 AND EPHB2 DIFFERENTIALLY CONTRIBUTE TO CRC PATIENT-DERIVED-TUMOR-ORGANOIDS DYNAMICS

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BACKGROUND-AIM

Patient Derived Tumor Organoids (PDTO) are 3D structures known to mimic closely the heterogeneous cyto-architecture of the originating specimens. We have shown the existence of distinct, EpHA2 and EphB2 expressing cell subpopulations in murine and human CRC samples. EphB2 is known to promote stem-like features in normal and diseased tissues including colon, and EphA2 may contribute to establish a stress resistant, EMT-like phenotype. Activation of stem-like cells, EMT-driven migration and stress resistance may play a role in organoid formation.

METHODS

PDTO cultures setting and propagation, flow cytometry analysis of EphB2pos and EphA2pos cell subpopulations and live/dead cell staining. RNAseq and QRT-PCR to assess the levels of EphA2 and EphB2 in CRC specimens and matched PDTOs.

RESULTS

We assessed any correlation between the expression levels of EpHA2 and EphB2 and the propensity of the primary tumors to grow as organoids. This revealed a 1.4 fold increase of EpHB2 mRNA in organoid growing tumors as compared to non-organoid growing ones (n=16, p<0.05) and a non significant yet similar trend in EpHA2 levels (p>0.05). We treated a CRC-derived PDTO culture with clinically-relevant doses of 5-FU (2.5ugr/ml, 96hrs), a still used chemotherapy for advanced CRC. FACS analysis revealed a steady increase of EpHA2pos cells and persistence of EpHB2 cells paralleled by a reduction in volume and diameter of the treated OGs over time, matching known cytostatic and cytotoxic effects of the 5-FU. This was confirmed by a reduced number of live EPHA2neg cells, as assessed by FACS after disaggregation. The percentage of EphA2pos cells persisted in chronically stressed PDTOs. We then evaluated whether EpHA2pos cells could maintain a stress resistant phenotype over time in chronically 5-FU challenged PDTO cultures (2.5ugr/ml, 21dd). When compared to vehicle treated controls (saline) no statistically significant decrease of EphA2pos cells was observed in three out of four treated cultures. However, a trend could be identified among the persistence of PDTOs after 5-FU-induced stress and the persistence of EpHA2pos cells within the disaggregated cultures.

CONCLUSIONS

The increased mRNA levels of EphB2 in organoid forming tumors reminds of the role of EphB2 in crypt development and the persistence of EpHA2pos cells in chronically treated CRC PDTOs matches what is emerging on the role for EpHA2 signaling at mediating adaptive response to stress. However, this POC study may need validation on a larger cohort of samples.

THE MULTI-OMICS BASED IMMUNE LANDSCAPE OF BRCA1/2-POSITIVE AND NEGATIVE MALE BREAST TUMORS.

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BACKGROUND-AIM

Compared with female breast cancer, male breast cancer (MBC) is rare and displays biological and clinical peculiarities. Gender-specific data on predictive molecular biomarkers are lacking, thus cutting out male patients from targeted treatments.

Here, we characterized the multi-omics immune landscape of MBC, in order to provide insights into immune-genetic properties of MBCs in relation to BRCA status and pathological features, that may help improving the selection of patients responsive to immunotherapy.

METHODS

Genomic and transcriptomic data of 66 male breast tumor samples, including 20 with germline BRCA pathogenic variants, were obtained by gene panel sequencing and RNA-sequencing.

Tumor mutational burden (TMB), Microsatellite Instability (MSI), PD-1 and PD-L1 gene expression, immune scores, and immune cell infiltration profiling by ESTIMATE and CIBERSORT were evaluated, as well as associations with pathology data and overall survival.

RESULTS

Overall, MBCs showed low levels of TMB, MSI and immune scores. High TMB (>10 mut/Mb) and high MSI (>10%) levels were reported in 6 (9%) and 10 (15%) MBCs, respectively. TMB or MSI values were not correlated with immune scores and with PD-1 or PD-L1 gene expression. Transcriptome-based immune cell profiling showed that CD4+ memory resting T cells, macrophages M0 and M2 represented the top three highest infiltrating fractions in MBCs (27%, 18% and 12%, respectively). Compared with non-BRCA MBCs, BRCA-associated MBCs showed higher TMB values (p=0.04), a higher fraction of CD4+ memory activated T cells (p=0.04), a lower fraction of activated mast cells (p=0.0003) and of eosinophils (p=0.004). Estrogen receptor (ER) negative status was associated with higher immune scores (p=0.008) and higher TMB values (p=0.036) compared with ER positive status. Among the biomarkers analyzed, high TMB was associated with a worse overall survival (p=0.0001).

CONCLUSIONS

Subsets of male breast tumors may present immunogenic characteristics.

The multi omics-based evaluation of immune-related biomarkers, matched with germline genetic profiling, may open to future characterizations of actionable strategies suitable for men with breast cancer.

Study supported by AIRC (IG21389) to LO.

P027

LIPID SIGNALING MODULATES MALIGNANT MESOTHELIOMA RESISTANCE. AN EX VIVO STUDY WITH PATIENT DERIVED MESOTHELIOMA ORGANOIDS (PDMOS)

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BACKGROUND-AIM

Tumor resistance to chemotherapy is accompanied by lipid metabolic reprogramming, an emerging cancer hallmark. There is an urgent need to understand the relationship between lipid metabolism and tumor biology, and to characterize lipids as prognostic biomarkers in cancer. We have recently shown that arachidonic acid (AA) is specifically released by malignant pleural mesothelioma (MPM) cells in vitro (and not by their un-transformed counterparts) after challenge with chemotherapy, and that the released AA signals chemoresistance through activation of the NFκB pathway (Cioce M, JECRC 2021). cPLA2 is chiefly responsible for the release of AA and we here demonstrate that a clinically validated cPLA2 inhibitor (AVX001) may attenuate MPM chemoresistance in vitro.

METHODS

Setup of PDMO cultures; mass-spectrometry (MS) analysis of MPM patient pleural effusion for lipid composition with or without chemotherapy treatment; QRT-PCR for studying the levels of specific enzymes involved in lipidomic changes.

RESULTS

By analyzing pleural effusions of MPM patients (n=12) obtained from MesoBank UK, we have found that chemo-treated patients (compared to chemo-naïve) have i) increased levels of specific AA-derivatives by MS and ii) a modulation of hub enzymes mRNA levels important for AA metabolism. Since we have previously shown that AVX001 a clinically available cPLA2 inhibitor, attenuates AA release and NFκB –driven chemoresistance in vitro, the similarity between our unpublished in vitro observation and the scenario reported in the MPM patients may be of clinical relevance. Our lab has set-up Patient Derived Tumor Organoids cultures from MPM patients (PDMOs) by employing specific media and conditions. We present here the results of targeting specific enzymes in MPM PDTOs (n=5) and the effect of inhabiting those latter with clinically approved drugs on the number, size and cell subpopulation composition of the treated PDMOs, before or after challenging those cultures with first line drugs.

CONCLUSIONS

Release of specific lipid species into the extracellular milieu is a feature of chemotherapy treated mesothelioma, in vitro and in vivo. Patient-Derived Mesothelioma-Organoids are currently employed to dissect and understand this phenomenon and its translational relevance. This investigation paves the way for novel insights in the metabolic reprogramming of chemo-resistant MPM.

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 glycolysis. 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 (MB), a tumor characterized by a reprogramming of energy metabolism toward aerobic glycolysis.

METHODS

Through structure-based virtual screenings, we identified potential LDHA inhibitors and we 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 anticancer activity that was lost in cells lacking LDHA, demonstrating its specificity. Therefore, 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.

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.

P030

PLASMA TRANSTHYRETIN AND RETINOL BINDING PROTEIN IN CARDIAC AMYLOIDOSIS: ANALYTICAL EVALUATION OF THE IMMUNONEPHELOMETRIC METHOD AND ELECTROPHORETIC STUDIES OF THEIR PATTERN

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BACKGROUND-AIM

Transthyretin cardiac amyloidosis (ATTR) is caused by extracellular deposition of transthyretin (TTR), a plasma carrier of thyroxin and retinol-binding protein (RBP). Aims of this study were: to evaluate the analytical characteristics of the nephelometric method used to quantify TTR and RBP; to develop a native electrophoretic method to characterize plasma TTR forms.

METHODS

Paired serum (S) and plasma lithium heparin (Li-P) samples (n=100) from healthy subjects and patients with ATTR were collected. Total TTR and RBP were quantified by nephelometry (Atellica, Siemens) according to the CSLI EP5-A2 protocol. Additional Li-P samples from patients with wild-type ATTR (n=10) were collected before (T0) and during (T90) tafamidis treatment. Samples were separated on a native 4–20% Tris-Gly polyacrylamide gel associated with western blot for TTR and RBP.

RESULTS

No differences were observed in the nephelometric measurement of TTR and RBP between paired S and Li-P samples. Reproducibility analysis showed similar analytical performances for nephelometry in both matrices (CV% intra-assay Li-P and S: TTR 1.9 and 2.5; RBP 2.3 and 1.5; CV% inter-assay: TTR 2.4 and 2.9; RBP 2.3 and 2.1).

According to electrophoresis, in all subjects TTR was present as: dimers or trimers, tetramers complexed with 1 (75kDa) or 2 RBP (100kDa), and high molecular weight aggregates (>150kDa). Based on TTR electrophoretic pattern at T0, patients could be divided in 2 groups characterised by the presence (Attr-1) or the absence (ATTR-2) of TTR tetramers. The ATTR-1 group showed higher levels of total TTR in comparison with ATTR-2 (mean±SD 25.5±3.8, 16.6±4.2 mg/dl, p=0.004), while RBP levels were similar (5.1±0.7, 5.1±1.4 mg/dl). The electrophoretic patterns, TTR and RBP levels of ATTR-1 were comparable to those of controls (TTR 22.6±3.8, RBP 4.4±0.9 mg/dl). Tafamidis treatment was associated with a progressive increase of total TTR levels in both groups (T90 31.4±5.8, 28.7±6.8 mg/dl).

CONCLUSIONS

Quantification of TTR and RBP by nephelometric assay could be performed both on S and Li-P samples with high reproducibility. Further studies will clarify if the electrophoretic pattern of TTR could represent a tool to assess the individual pharmacological response to tafamidis.

P031

TARGETED INHIBITION OF THE METHYLTRANSFERASE SETD8 SYNERGIZES WITH THE WEE1 INHIBITOR ADAVOSERTIB IN RESTRAINING GLIOBLASTOMA GROWTH IN VITRO AND IN VIVO

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BACKGROUND-AIM

Glioblastoma multiforme is a largely incurable tumor, with a dismaying median survival time of 15 months. Despite intense searching, very few progresses have been made in the individuation of new effective drugs.

The lysine methyltransferase SETD8 is overexpressed in many solid and hematological human tumors, its oncogenic role strongly suggested by the evidence that SETD8-induced methylation of the Proliferating Cell Nuclear Antigen (PCNA) protein promotes cancer cell proliferation. Of note, there are also numerous indications that, by methylating other substrates than PCNA, the effects of SETD8 on cancer cells might be pleiotropic and tissue-specific.

To date, there are no data about levels of expression and function of SETD8 in glioblastomas

METHODS

We tested SETD8 expression by IHC and RNA seq analysis. All experiments were performed on different glioblastoma cell lines (U251, T98, LN18 and SW1088) and on primary isolated glioblastoma cells.

DNA damage was evaluated by immunofluorescence using p-H2Ax. Cell cycle arrest was monitored with FACs and western blots analyses.

Cell viability was assessed using MTT test and apoptosis with caspase activity assays.

Xenograft experiments were performed by inoculating luciferase-positive U251 cells.

RESULTS

Our evidence proved a functional role for SETD8 in glioblastoma. A specific SETD8 inhibitor, UNC0379, blocks glioblastoma cell line proliferation, by inducing DNA damage and, in turn, safeguarding activation of the G2/M cell cycle checkpoint. 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 data indicate the association UNC0379+AZD1775 as a successful strategy for glioblastoma treatment, especially for those overexpressing SETD8.

CONCLUSIONS

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

We found the efficacy of drug combination, also in xenograft model. In conclusion, based on the all above considerations, our preclinical data encourage further trial of the novel UNC0379+adavosertib combinatory approach for glioblastoma treatment.

P032

GLUTAMINE REGULATES BCR/ABL EXPRESSION IN HYPOXIC CHRONIC MYELOID LEUKEMIA CELLS VIA FATTY ACIDS METABOLISM

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BACKGROUND-AIM

Under very low oxygen tension, Chronic Myeloid Leukemia (CML) cells undergo the suppression of the BCR/Abl oncoprotein, whereas a BCR/Abl-independent subset of cells, commonly referred to as leukemia stem cells, is maintained. Such cell population retains the capacity, when transferred to normoxic conditions, to generate a BCR/Abl-expressing progeny which is, in vivo, responsible for the relapse of the disease, demonstrating to be also resistant to the tyrosine kinase inhibitors (TKi) by lacking their molecular target. Moreover, under oxygen restriction, glutamine plays a major role, stabilizing c-Myc expression and inducing cancer cells to diverge towards a more pronounced fatty acids (FA) metabolism.

METHODS

K562 and KCL22 cell lines were subjected to glucose and/or glutamine deprivation in hypoxic conditions. Cells metabolic profile was assessed through the Seahorse XFe96 while L-Glutamine-¹³C₅ was exploited via LC/MS to determine its contribution to FA de novo synthesis. BODIPY 493/503 was used to measure the intracellular neutral lipid droplets in confocal microscopy and flow cytometry. BCR/Abl was evaluated via Western Blotting whilst CD36 was determined through flow cytometry.

RESULTS

We observed that glutamine is capable to boost glycolysis, leading to a faster BCR/Abl downregulation in hypoxic conditions, and decrease the basal and maximal cell respiration capacity. We also identified that under oxygen and glucose shortage, CML cells were characterized by numerous lipid droplets. Such an augmented neutral lipid content was due to a glutamine-dependent CD36 upregulation, which is able to uptake FA from the extracellular milieu. In these conditions, CML cells rapidly lose BCR/Abl expression, a phenomenon which was validated by the treatment with exogenous BSA-Palmitate, while the use of the sulfosuccinimidyl oleate, a specific CD36 inhibitor, sustained the oncoprotein maintenance instead.

CONCLUSIONS

Our results suggest that FA may play a fundamental role in hypoxic-induced BCR/Abl suppression and that FA degradation might be needed for the oncoprotein re-expression once normoxic conditions are restored. This phenomenon might be therefore exploited to sustain BCR/Abl expression in hypoxic cells to be susceptible to TKi.

P033

"BREAST CANCER MULTI-OMICS CHARACTERISATION POINTS TO METABOLIC FEATURES AS ACQUIRED TRAITS OF RESISTANCE"

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BACKGROUND-AIM

In breast cancer (BC), metabolic reprogramming plays an important role not only in carcinogenesis but also in pharmacological resistance mechanisms. Tamoxifen is the first-line medication in post-surgery adjuvant therapy in BC due to its effectiveness and safety. Despite its efficacy, 20-30% of cancers are resistant to tamoxifen therapy, either as de-novo resistance or as acquired resistance during treatment. Recent research has focused on targeting metabolism to find novel therapeutic targets for BC in clinical practice and overcome medication resistance.

METHODS

FASN and LDHA, two different genes involved in cellular respiration and glycolysis, were investigated for expression and methylation profiles in BC patients and healthy tissues, as well as their correlations to tamoxifen resistance therapy, using TCGA and publicly available datasets (UALCAN). IHC data from 104 BC samples support the unfavorable relationship between FASN and LDHA in terms of tumor aggressiveness. Furthermore, MCF-7 and MCF-7 TamR were employed as in vitro models of Lum A and Lum B subtypes, reflecting respectively their sensitivity or resistance to tamoxifen.

RESULTS

This study demonstrates FASN and cell respiration are primary metabolic sources in sensitive conditions, and how their downregulation is linked to high tumor grade and reduced life expectancy. Oppositely, LDHA overexpression and glycolysis are tamoxifen resistant metabolic features, strongly connected with tumor grade and worse prognosis.

CONCLUSIONS

These data suggest how metabolism can be considered as further potential therapeutic approach for cancer therapy. Different metabolic targets can be identified and modulated by specific inhibitors (mostly in use in clinical settings for non-cancer purposes). The metabolic approach may be useful in treating aggressive and resistant cancers.

P034

THE LONGEVITY ASSOCIATED VARIANT IN BPIFB4 TARGETS PLATELETS INDUCING ENDOGENOUS PROTEIN RELEASE AND ITS FAVORABLE HEALTH OUTCOMES

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BACKGROUND-AIM

Beyond their role in haemostasis and thrombosis, activated platelets release a lot of mediators that support a modulatory role in mono-macrophage compartment in the context of vascular disease and inflammatory states. BPIFB4 belongs to a family protein participating in the innate mechanism of host defense and its Longevity-Associated Variant (LAV) has proved strategic to cope with frailty conditions, aging-related events, e.g. cardiovascular ones, and immune dysfunction mainly through a favorable conditioning of macrophages.

METHODS

The aim of this study was to characterize the expression of BPIFB4 in human platelets and megakaryocytes. We used PrP and the human megakaryoblastic cell line MEG-01 as an in vitro model and tested the involvement of platelets in the immune modulatory role of the BPIFB4 protein and in the functional effects of the LAV-BPIFB4 polymorphic variant by injecting a platelet blocking antibody anti-CD42b (anti-Plts) or a control antibody (veh) 4 days after AAV-LAV-BPIFB4 gene delivery in a mouse model in vivo. Finally, we describe rhLAV-BPIFB4 inhibitory effects on platelet aggregation in vitro and an antithrombotic activity in vivo

RESULTS

Here, we reveal that platelets are critical to boosting the mono-macrophages polarizing activity of recombinant (rh)LAV-BPIFB4 both in vitro and in vivo where platelet depletion completely abolished the vasculo-protective action of LAV-BPIFB4 and suppressed its pro-resolutive CD206+anti-/CD86+pro-inflammatory Ly6C+ monocyte skewing to LPS stimulation.

Further, we discovered that platelets themselves are a reservoir of BPIFB4 protein secreted upon a positive feed-forward mechanism. Indeed, in vitro, BPIFB4 was released by calcium activated human platelets, a process that is amplified in LAV allele carriers donors. Furthermore, in vivo, LAV gene transfer in mice resulted in an enhanced protein enrichment in bone marrow megakaryocyte precursor cells and in vitro, in a MEG-01 megakaryoblastic cell line, in an improvement of platelet-like particles production.

From a translational point of view, rhLAV-BPIFB4 treatment showed a reduced platelet-platelet aggregation that was also reflected by protection from a lethal pulmonary thromboembolism in mice.

CONCLUSIONS

In summary, our data indicate that BPIFB4 is carried by platelets, which are functional for LAV activity and that rhLAV-BPIFB4, in agreement with its protective role, can also counteract platelet hyperactivation and haemostasis related events.

P035

FAD DEPENDENT LSD1 FACILITATES METABOLIC DYNAMICS IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

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BACKGROUND-AIM

A growing body of research has established LSD1 (KDMA1) in dual biological settings as an epigenetic target as well as a system for using energy expenditure in the cancer. According to findings, blocking of LSD1 shrinks tumor growth, glycolytic performance and activates mitochondrial respiration by triggering energy expenditure and mitochondrial metabolic genes. Our particular interest lies in the metabolic shift mediated by LSD1 in pancreatic cancer, in which the FAD dependent LSD1 suppresses mitochondrial metabolism, while promoting glycolysis under oxygen conditions. In order to better understand the synergistic potential and plasticity of LSD1 dynamics in epigenetics and metabolic re-programming, ORY-1001 was applied to an in vitro PDAC system.

METHODS

The potency of ORY-1001 as a LSD1 selective inhibitor was evaluated in four pancreatic cancer cell lines: PL-45, SW1990, PANC1 and HPAF II. As part of our experiments, we assessed cell proliferation, cell death, the cell cycle, colony formation, and in vitro migration of cells. An evaluation of protein expression for different metabolic targets was carried out in PL-45 cells that were treated with ORY-1001. Glycolytic rate assays and Mito-stress kits were also used to assess metabolic status.

RESULTS

During our investigations, we found that LSD1 selective inhibitor ORY-1001 leads to cell death and cell cycle arrest and thereby reducing the transition into S/G2M phases. A similar tendency was observed in the form of reduction in colony formation potential and cell migration at both concentration of ORY-1001 (100uM and 200uM). Accordingly, inhibiting LSD1 leads to excessive fatty acid oxidation inside mitochondria, leading to increased ROS levels, mitochondrial damage, and apoptosis. To confirm whether reduced viability, increase in cell death and cell cycle arrest is due to the decrease in glycolysis, we performed glycolytic rate assay and mito-stress assay to investigate the LSD1 inhibition role in both cellular respiration and glycolysis. Inhibition of LSD1 in PL-45 and SW1990 cell lines significantly reduced oxidative phosphorylation than glycolysis, and higher doses reduce mitochondrial respiration.

CONCLUSIONS

According to our findings, inhibiting LSD1 influence mitochondrial performance more than glycolytic pathway function, as revealed by metabolic and protein expression analyses.

OVERCOMING DRUG RESISTANCE IN GASTRIC CANCER: CARBONIC ANHYDRASE IX AS A MOLECULAR TARGET TO BOOST THERAPY RESPONSE AND STIMULATE IMMUNOGENIC CELL DEATH

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BACKGROUND-AIM

Gastric cancer (GC) is the fifth most frequently diagnosed malignancy and the fourth leading cause of cancer-related death worldwide. When surgery is not pursuable, multimodal perioperative chemotherapy (pCT) is used to improve patients' OS. However, GC progressively gains chemoresistance, limiting therapies, thereby the identification of suitable targets to overcome drug resistance is of fundamental interest. In this context, the carbonic anhydrase IX (CAIX) has gained the most attention.

METHODS

GC patients who underwent pCT FLOT (i.e., Leucovorin, 5-Fluoracil, Docetaxel, and Oxaliplatin) followed by surgery in the Careggi Hospital in Florence were classified as responder and non-responder, depending on the tumor regression grade, and CAIX was evaluated by IHC in FFPE GC sections. pCT FLOT was administered in vitro to CAIX-high and CAIX-low-expressing GC cells, previously sorted at FACS within the AGS population, or to AGS forced to over-express CAIX. FLOT-resistant GC cells were generated according to the "high-level laboratory models". SLC-0111 was used to inhibit CAIX activity in GC cells. Colony formation, apoptotic assays, and GC spheroids growth were evaluated following GC cell treatment with SLC-0111/FLOT. The expression or release by GC cells of DAMPs as Annexin A1 (ANXA1), Calreticulin (CALR), and High Mobility Group Box 1 (HMGB1) was evaluated in flow cytometry and ELISA following the SLC-0111 treatment.

RESULTS

CAIX was over-expressed in non-responder GC patients than in responders. CAIX-high-expressing AGS GC cells were more resistant to the pCT FLOT than the CAIX-low-expressing counterpart, and CAIX-overexpressing cells showed a significantly impaired therapy response. FLOT-resistant GC cells over-expressed CAIX compared to control cells. SLC-0111 significantly improved the therapy response of both wild-type and resistant GC cells, being also able to induce in GC cells a death with immunogenic potential, as suggested by the increased level of CALR, ANXA1, and HMGB1.

CONCLUSIONS

Overall, these data suggest a correlation between CAIX and GC drug resistance highlighting the efficacy of SLC-0111 in boosting the therapy response of GC cells, in re-sensitizing resistant GC cells to pCT, and potentially in warming up the tumor by inducing immunogenic death of GC cells.

P037

DNA METHYLATION SIGNATURES DISCRIMINATE INFLAMMATORY STATUS OF THE INTESTINE IN CHILDREN AFFECTED BY AUTISM SPECTRUM DISORDERS

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BACKGROUND-AIM

It has been well elucidated the connection existing between gut and brain and several ways have been identified allowing the communication between the two organs. One of the pathways connecting brain and gut may be the possibility that some gut inflammatory markers may reach the brain through blood inducing neuroinflammation. To date, it is well-defined the presence in ASD patients of both a neuroinflammatory component and widespread intestinal inflammation, with alteration of the gut microbiota and the presence of the so-called "leaky gut". In this context, epigenetic signatures at genes involved in the inflammation may play an important role in the alteration of the inflammatory pathways. Thus, we wondered whether DNA methylation may mark gut inflammation in children affected by ASD compared to healthy age- and sex-matched children.

METHODS

We extracted DNA from stool of 7 healthy children and 8 children at first diagnosis of ASD in order to avoid biases due to drug assumption aged between two and four years. We used DNA from stool that may be considered a surrogate of epithelial and inflammatory intestinal cells. We performed Illumina EPIC array, interrogating the methylation state of 850,000 CpG sites per sample. Microbiota composition was evaluated by analyzing the rRNA 16S by Next Generation Sequencing.

RESULTS

We identified 438 differentially methylated genes the majority of which belonged to inflammatory pathways, such as TLR3, IL6, INFGR2. We identified also genes potentially involved in the biosynthesis of SCFA. We found DNA methylation changes at MLYCD that catalyzes the conversion of malonyl-CoA to acetyl-CoA, a precursor of butyrate compound. Finally, we found that children affected by ASD presented a statistically significant higher epigenetic age compared to non-affected children. This finding well correlated with the "microbiotic age" of ASD affected children, since the gut microbiota composition in these children showed increased levels of some bacterial species commonly found in the adulthood.

CONCLUSIONS

Our data demonstrated that: 1) methylome analysis of stool in ASD children discriminates the disease, especially at inflammatory genes; 2) DNA methylation at specific genes potentially impacts on the production of SCFAs, the metabolic counterpart of gut microbiota; 3) ASD children showed increased epigenetic and microbiotic age. Our approach may be useful for the identification of the ASD associated epigenetic signatures and may be potentially extended to other inflammatory bowel disease.

P038

DISCOVERY AND FUNCTIONAL CHARACTERIZATION OF NATURALLY INSPIRED INHIBITORS OF THE MITOCHONDRIAL FISSION PATHWAY: PRECLINICAL EVIDENCE IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Defects in mitochondrial fission, leading to increased number of small and dysfunctional mitochondria, have been reported to impact tumorigenesis. The main regulator of such process, i.e. the GTPase Drp1, plays an oncogenic role in various cancer types, including multiple myeloma (MM). The repertoire of currently available Drp1 inhibitors is very limited.

We here describe an experimental workflow leading to the discovery of naturally inspired candidate inhibitors of Drp1 in MM models.

METHODS

Molecular docking was performed on the crystallographic structure of Dynamin-1-like protein (Drp1) corresponding to PDB entry 4H1V. Cell viability was assessed by Cell Titer Glo assay. Lipid peroxides were assessed by BODIPY C11 FACS-analysis; apoptosis was determined by Annexin-V staining. Ion AmpliSeq™ Transcriptome Human Gene Expression Core Panel was used for the analysis of MM transcriptome changes.

RESULTS

Based on previous studies indicating that certain polyphenols can inhibit Drp1 we focused on Hesperetin (Hes) and Naringenin (Nar), both highly abundant in extracts of Tacle®, a citrus variety typical of our region. Re-docking experiments were performed to calculate the binding energy value for the crystallographic ligand GNP into Drp1 binding site; next, Hes and Nar were docked into Drp1, and both compounds found accommodating in the binding site with favourable binding energy values, and capable of interacting with the same residues previously involved in the interaction with synthetic Drp1 inhibitors.

Hes and Nar increased the number of fused mitochondria, as observed by transmission electron microscopy, and phenocopied Drp1 genetic and pharmacological inhibitors, decreasing MM cell viability and clonogenicity, triggering ferroptosis and apoptosis, and synergizing with proteasome inhibitors. Mechanistically, Hes and Nar treatment produced transcriptomic perturbations in MM cells which overlapped those produced by known synthetic Drp1 inhibitors, including the down-regulation of lipogenesis-related transcription factors (c-MYC, SREBF1/2), found associated to a decrease in total triglycerides and cholesterol. All the above-reported molecular and functional effects were also demonstrated after treatment of MM cells with Tacle juice extract.

CONCLUSIONS

These findings suggest that Hes and Nar act by targeting aberrant mitochondrial dynamics in MM, and could represent suitable leads for developing novel Drp1 inhibitors.

P039

SINGLE CELL AND SPATIAL TRANSCRIPTOMICS REVEAL METABOLIC HETEROGENEITY IN PANCREATIC CANCER

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BACKGROUND-AIM

The incidence of pancreatic ductal adenocarcinoma (PDAC) has increased in recent years with a 5-year survival rate of about 9%. The major causes of treatment failure include late detection and aggressive biology, stressing the need for new therapeutic approaches and specific biomarkers. Anabolic and catabolic processes in PDAC are involved in cellular functions such DNA duplication, redox reactions, and overall homeostasis in addition to being primarily energetic. According to the evidence for a particular metabolic phenotype of PDAC, it is conceivable to reduce the aggressiveness of this tumor by targeting cellular bioenergetic circuits. The study aims to identify highly malignant cell subpopulation(s) in PDAC that displayed a high metabolic rate as the key feature to obtain glycolytic/lipolytic markers for PDAC early diagnosis by employing single cell technology and spatial transcriptomic.

METHODS

By using published database of single cell PDAC patients, we identified a metabolic signature in tumor subpopulations. Then we used this signature to validate metabolic dependency in a preserved tumoral architecture with spatial transcriptomic in our cohort of PDAC patients. Finally, we moved in in vitro systems to corroborate the possibility to interfere with metabolic signaling in PDAC cellular models.

RESULTS

PDAC cells exhibited extensively reprogrammed metabolism to meet their energetic and biomass demands under extremely harsh conditions. The metabolic alterations were tightly related to the signaling that was brought on by oncogene activation and tumor suppressor inactivation, indicating an energy adaptability reflecting tumoral architecture and structural organization.

CONCLUSIONS

Metabolic control of PDAC cells is crucial to progression in systems with a high rate of proliferation. We show metabolic dependency driving pancreatic cancer heterogeneity.

P040

PD-L1 OVEREXPRESSION INDUCES ANGIOGENIC CYTOKINE SECRETION AND ENDOTHELIAL CELL MIGRATION VIA STAT SIGNALING.

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BACKGROUND-AIM

PD-L1 is frequently overexpressed in many types of human cancers, including NSCLC, and is associated with a poor prognosis. Immunotherapy has significantly changed the treatment landscape for advanced NSCLC with the introduction of drugs targeting PD-1 and PD-L1. Recent evidence showed that pro-angiogenic factors have immunosuppressive activity and the combination of anti-angiogenic agents with immunotherapy has been evaluated in the treatment of several cancers. However, the correlation between PD-L1 overexpression and angiogenesis has not been yet elucidated.

METHODS

Membrane PD-L1 expression was detected by confocal microscope and flow cytometry. Intracellular signalling pathways were analyzed by RNAseq, phospho-proteome assay and western blotting. Angiogenic cytokine secretion was determined by Luminex. Huvec migration was carried out by the transwell system.

RESULTS

We generated two NSCLC cells (A549 and H460) with high PD-L1 expression. PD-L1 overexpression affects inflammatory and JAK/STAT signaling, compared to parental cells. It has been reported that the STAT pathway can control tumor angiogenesis and it is mainly activated by cytokine secretion from immune cells. By TCGA data bank correlation analysis, PD-L1 expression is positively related to VEGF A, C, and D levels. Co-culturing tumor cells with low/high PD-L1 level with PBMC, a higher release of pro-angiogenic cytokines was observed in the presence of PDL1 overexpressing cells. The JAK/STAT inhibitor Ruxolitinib significantly reduced the secretion of the pro-angiogenic factors, suggesting that their production is a consequence of STAT activation. Huvec cell migration was enhanced in the presence of higher cytokine levels, and the anti-angiogenic drug Nintedanib's addition prevents Huvec cells migration.

CONCLUSIONS

We demonstrated that cells with high PD-L1 levels produce more pro-angiogenic cytokines via STAT signaling. These cytokines stimulate Huvec cells migration that is reverted by Nintedanib. The combination of an anti-angiogenic agent with an immune checkpoint inhibitor in patients with high PD-L1 could represent a promising pharmacological option.

P041

THE NICHE MICROENVIRONMENT AS A CRUCIAL FEATURE IN CML LSCS STEM CELL POTENTIAL AND QUIESCENCE MAINTENANCE.

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BACKGROUND-AIM

Chronic Myeloid Leukaemia (CML) is a stem cells-driven myeloproliferative disease which occurs in haematopoietic stem cells due to the reciprocal t(9;22) translocation. Despite paramount steps forward in CML treatment, current therapeutic strategies are not curative yet. Certainly, features of the bone marrow stem cell niche (SCN) environment, such as oxygen tension, cytokines, adhesive proteins, play a crucial role in maintaining Leukaemic Stem Cells (LSC) and sustaining their escape from the effects of therapy. Our work was directed to unravel LSC metabolic features and interaction with the endosteal niche.

METHODS

K562 and KCL22 stabilized CML cell lines were incubated in atmosphere at 0.1% oxygen inside a gas-tight manipulator/incubator. At the end of such a selective incubation, cells were lysed to assess BCR/Abl expression and signalling or transferred into non-selective cultures incubated in air, enabling cell culture expansion, to evaluate the maintenance of stem cell potential at the end of selective incubation. Furthermore, CD34+/CD38-, BCR/Abl-expressing CML cells of the TF1-BA line, manipulated to bear the FUCCI system (a dynamic way to track cell cycle), were seeded within a bone marrow-like 3D culture system (based on a stromal and an endothelial cell lines) to determine whether LSC dormancy is long-term fostered by either cell adherence to the endosteal niche and secretion of Bone Morphogenetic Protein (BMP).

RESULTS

Low oxygen tension led LSC to lose BCR/Abl protein expression, thereby rendering them un-targetable by TKI. This phenomenon, mainly dependent on glucose consumption, in turn is critically governed by glutamine availability within the SCN. Cells rescued from low oxygen selective cultures repopulate secondary expansion cultures following BCR/Abl re-expression and after a lag-phase of variable length, depending on the overall metabolic conditions in selective cultures. On the other hand, we found that BMP4 promotes the rapid commitment of LSCs to a deep quiescent state, which is enhanced under TKI treatment. Moreover, the 3D culture system provided consistent data regarding a block of TKI-treated cells in G1 and a significant decrease of cell transit in the G2-M cell cycle phases, with a greater effect of Nilotinib.

CONCLUSIONS

The loss of LSC reliance upon BCR/Abl expression and signalling, alongside the commitment to quiescence, are key events involved in both cells' refractoriness to CML treatment and persistence within the SCN environment.

CYTOKINE-INDUCED INOS IN ALVEOLAR EPITHELIAL CELLS: COOPERATIVE EFFECTS OF JAK/STAT AND NF-KB

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BACKGROUND-AIM

The respiratory tract is the main pathological site of SARS-CoV-2 infection, where an uncontrolled immune response can worsen lung damage. Recent evidences point to the expression of inducible nitric oxide synthase (NOS2/iNOS) as a component of the inflammatory response in severe COVID-19 patients. Here we address the effects of cytokine-enriched conditioned medium from Spike-activated macrophages on iNOS expression in lung epithelial cells, as well as the molecular pathways involved.

METHODS

Human macrophages derived from circulating monocytes were incubated with Spike S1 from SARS-CoV-2 for 24 h; the resulting conditioned medium (CM_S1) was analyzed for cytokines content with Elisa Assay and found enriched of inflammatory mediators including INF_{γ} , TNF_{α} , IL-1 β and IL-6. A549 lung cells were, then, exposed to CM_S1 or to 50 ng/ml INF_{γ} , TNF_{α} , IL-1 β and IL-6, alone or in combination. iNOS expression was assessed with RT-qPCR and Western blot; nitric oxide (NO) production was determined through the measurement of nitrites in the incubation medium. An IRF1 knockout A549 cell line (IRF1 KO) was used to study the molecular pathways underlying iNOS induction, along with 1 μ M baricitinib and 20 μ g/ml CAPE, inhibitors of JAK/STAT and NF- κ B, respectively.

RESULTS

In A549, the exposure to CM_S1 significantly induces the expression of NOS2. INF_{γ} , TNF_{α} and IL-1 β all stimulated NOS expression, either alone and, even more, when combined; IL-6 was, instead, ineffective. The same pattern of expression was observed in IRF1 KO cells, although at very lower levels compared to WT cells; consistently, a significant increase of NO production was observed in WT, but not in IRF1 KO, upon simultaneous incubation with the three cytokines. The induction of NOS2 by CM_S1, which was much lower in IRF1 KO than in WT cells, was suppressed by baricitinib and partially prevented by CAPE in both models.

CONCLUSIONS

These findings allow to draw a model for CM_S1-mediated induction of iNOS, where cytokines secreted by S1-activated macrophages, mainly INF_{γ} , IL-1 β and TNF_{α} , activate both IRF1 and NF- κ B through the JAK/STAT1 pathway. Our data further sustain the therapeutic efficacy of baricitinib in COVID-19, since, beside limiting cytokine storm, it also prevents iNOS induction.

P043

DECIPHERING MOLECULAR LANDSCAPE OF CARDIAC MYXOMA BY MULTI-OMICS

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BACKGROUND-AIM

Cardiac myxoma (CM) is a common primary neoplasm of the heart, that despite being biologically benign represents a life-threatening condition due to high embolization potential. The majority of CMs arise sporadically and only 3-10% of cases occur in the setting of Carney complex, an autosomal-dominant disorder arising as a consequence of inactivating mutations of PRKAR1A gene encoding for regulatory subunit of cAMP-dependent protein kinase type 1 α . Since little is known about molecular mechanisms underlying CM development, especially for sporadic cases, our study aimed to determine molecular hallmarks characterizing these rare tumors by means of next-generation sequencing technologies.

METHODS

Starting from an assumption that neoplastic cellular transformation, may be ignited by molecular alterations at multiple levels including genome, epigenome, and transcriptome, we applied a multi-omics approach by combining whole exome sequencing, CpG DNA methylation arrays, transcriptome, and miRNome profiling coupled with integrative data analysis aimed to dissect the molecular mechanisms behind CM pathogenesis.

RESULTS

Genomic profiling allowed to discriminate between tumors bearing wild-type and mutated form of the PRKAR1A allele and to detect the presence of pathogenic variants in other genes of the cAMP signaling pathway. Strikingly, transcriptional profiling revealed that inhibition of cAMP-dependent signaling occurs independently on the tumor genetic background pointing out an alternative way of its regulation. Interestingly, DNA methylation changes were identified in more than 30.000 CpG sites, but these alterations alone cannot explain the aberrant cAMP activity. miRNome analysis instead revealed the presence of many deregulated miRNAs that can control both cAMP and other cellular signaling involved in the neoplastic transformation.

CONCLUSIONS

These findings outline a complex molecular and functional landscape of CMs, highlighting novel players and possible disease biomarkers, worth to be exploited in the future.

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P044

NOVEL DIAGNOSTIC ASSAYS TO DISCRIMINATE ACTIVE FROM LATENT TUBERCULOSIS IN CHILDREN.

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BACKGROUND-AIM

In pediatric patients high accuracy diagnostic screening tests for tuberculosis (TB) are required to improve the diagnosis of both active TB and latent Mycobacterium tuberculosis (MTB) infection (LTBI). The novel IGRA LIOFeron TB/LTBI assay was tested in a pediatric setting and its accuracy was compared to the QuantiFERON-TB Gold Plus assay.

METHODS

A total of 87 pediatric subjects were enrolled: 13 patients with active TB, 16 with LTBI and 6 patients with Nontuberculous Mycobacteria (NTM) infection as control, since cross-reaction with some species of NTM is described with standard IGRAs. The blood of all the pediatric patients was tested with LIOFeron TB/LTBI assay, containing MTB Alanine-dehydrogenase, able to discriminate active TB from LTBI diagnosis. The results obtained with both IGRAs, performed on the same 87 samples, were finally compared.

RESULTS

Both IGRAs detected all the 51 (100%) healthy subjects as negative. Regarding QuantiFERON-TB Gold Plus test results, 23/26 (88.5%) patients with MTB infection had a positive result and 3/26 (11.5%) were identified as false negative (1/12 with active TB and 2/14 with LTBI). In LIOFeron TB/LTBI test 29/29 (100%) MTB infected patients had a positive response, with no false positives. None of the two assays resulted positive in NTM infected patients. Thus, the two IGRA assays demonstrated an excellent concordance of their results with patients' diagnosis of MTB infection. ROC analysis showed high accuracy in detecting both patients with active TB and LTBI for each test, with the Area Under the Curve > 0.9. LIOFeron TB/LTBI assay demonstrated a trend toward a higher sensitivity.

CONCLUSIONS

The two IGRAs displayed the same high accuracy in diagnosing MTB infection/TB disease, however LIOFeron TB/LTBI assay demonstrated higher sensitivity than QuantiFERON-TB Gold Plus test in LTBI detection.

P045

APPLICATION OF PATIENT-DERIVED LIVER ORGANOIDS FOR THE DEVELOPMENT OF NEW PERSONALIZED THERAPIES TARGETING VDAC1 IN INTRAHEPATIC CHOLANGIOCARCINOMA

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BACKGROUND-AIM

Intrahepatic cholangiocarcinoma (iCCA) is characterized by a very poor outcome, and reliable biomarkers as well as new therapeutic strategies are urgently needed. As a main actor in the regulation of mitochondria-mediated cell death and survival signaling pathways, Voltage Dependence Anion Selective Channel isoform 1 (VDAC1) became an attractive pharmacologic target. Many molecules have been conceived, however, due to promiscuity and side effects, none of them have been extensively used to treat patients.

The aim of this study was to test a new class of small molecules targeting VDAC1 to induce activation of the apoptotic pathway in iCCA patient-derived liver cells and organoids.

METHODS

To generate organoids, we minced tumor and paired non-tumor biopsies and shortly digested in small cell clusters that are seeded into Matrigel. After characterization using immunofluorescence and qPCR techniques, we treated primary cell cultures and organoids with different concentrations of small molecules targeting VDAC1, monitoring cell viability, to verify the *in vitro* effects and the efficiency of these compounds on cells.

RESULTS

We developed and established a biobank of human iCCA-derived organoids, evaluating the morphological characteristics and assessing a mathematical tool to model tumor growth. In addition, we analysed the presence of typical CCA markers (EpcAM, CK19, CK7, E-Cadherin, Ki67). We also investigated VDAC1 expression underlying higher levels in iCCA cells in comparison with non-tumor cells ($p < 0,005$).

We subsequently examined the efficiency of new small molecules targeting VDAC1, at different time points and concentrations, both in patient-derived cell cultures and organoids. In particular, we showed a significant decrease of viability in tumor cells only.

CONCLUSIONS

We developed and characterized a well-defined iCCA *in vitro* model that allowed us to investigate the effect of small molecules targeting VDAC1 as a new personalized therapy.

ROLE OF RDS 3337 HEPARANASE INHIBITOR ON CROSSTALK BETWEEN APOPTOSIS AND AUTOPHAGY IN HUMAN GLIOBLASTOMA CELLS

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BACKGROUND-AIM

Heparanase (HPSE) is an enzyme known as an endo- β -glucuronidase, which plays a significant role in cleaving heparan sulfate side chains. This enzymatic activity results in the disassembly of the extracellular matrix, ultimately promoting cell invasion and the dissemination of metastasis. In our research, we focused on examining the impact of a novel HPSE inhibitor, RDS 3337, on the regulation of the autophagic process as well as its influence on the delicate balance between apoptosis and autophagy in U87 glioblastoma cells. By studying the effects of this inhibitor, we aimed to gain insights into its potential as a therapeutic agent for modulating these critical cellular processes in glioblastoma.

METHODS

The investigation focused on analyzing the autophagy and apoptosis processes in human glioblastoma cells. Both untreated cells and cells treated with the benzazoyl derivative RDS 3337 were analyzed by immunoblotting and flow cytometry analysis. By combining the complementary strengths of these techniques, the study aimed to gain a comprehensive understanding of how the benzazoyl derivative RDS 3337 influences autophagy and apoptosis processes in human glioblastoma cells.

RESULTS

We observed, firstly, that LC3II expression increased in U87 cells incubated with RDS 3337, together with a significant increase of p62/SQSTM1 levels. These findings suggest an accumulation of autophagosomes and an inhibition of autophagic-lysosomal flux, indicating an impairment of the autophagic clearance. Conversely, the suppression of autophagic flux could activate apoptosis mechanisms, as revealed by the activation of caspase-3, the increased level of cleaved PARP1 and DNA fragmentation.

CONCLUSIONS

The findings of our study strongly support the concept that HPSE plays a significant role in promoting autophagy. These results provide compelling evidence that the novel HPSE inhibitor, RDS 3337, effectively blocks autophagic flux in the context of U87 human glioblastoma cells. This observation implies that the HPSE inhibitor has the potential to influence the delicate balance between apoptosis and autophagy in these cells, suggesting a potential role for this new class of compounds in the control of tumor growth progression.

P047

TARGETING EZH2 ACTIVATES NOTCH SIGNALING IN CERVICAL CANCER AND ACUTE MYELOID LEUKEMIA

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BACKGROUND-AIM

Unproper Notch pathway activation is involved in tumor pathogenesis by suppressing or promoting cancer, depending on tissue context. The molecular mechanisms sustaining Notch hyperactivation in cancer are well known, and several therapeutic approaches interfering with the signaling have proven antitumor efficacy. However, mechanisms underlying Notch repression have been poorly investigated in tumors in which its activation would be potentially relevant for cancer therapy. Recent studies have revealed a critical interaction between epigenetic regulators and Notch pathway activity. In particular, the balance between the activities of the demethylase JMJD3 and the methyltransferase EZH2 regulates Notch oncogenic transcriptional output in T cell acute lymphoblastic leukemia (T-ALL), indicating JMJD3 inhibition as a potential approach to face this malignancy. However, EZH2 oncogenic function over Notch regulation is still underexplored in tumors where the signaling is turned off, and its activation might suppress tumor growth.

METHODS

SiHa, HeLa, HL-60, and Me-1 cell lines; In vitro pharmacological treatment; Gene silencing; Trypan blue exclusion assays; RT-qPCR; Nuclear/cytosol fractionation; Western blotting.

RESULTS

Given JMJD3 and EZH2 function in regulating Notch oncogenic program in T-ALL, we speculated that this epigenetic-based system might be conserved in cervical cancer (CC) and acute myeloid leukemia (AML), in which the signaling functions as a tumor suppressor. Supporting our hypothesis, EZH2 inhibition activated Notch signaling and reduced viability of SiHa and HeLa CC, and HL-60 and Me-1 AML cells. Surprisingly, Notch blockage attenuated the EZH2-inhibition-dependent biological and molecular effects only in SiHa and HL-60, suggesting that these outcomes might be related to Notch activation in these specific tumor subtypes. In addition, by evaluating the EZH2/Notch axis in counteracting or prime drug resistance, we observed that EZH2 blockage potentiated cisplatin action in SiHa and HL-60.

CONCLUSIONS

Altogether, our results indicate that EZH2 oncogenic function partially encompasses Notch repression in AML and CC and propose the combination of EZH2 inhibitors with platinum-based drugs to enhance treatment efficacy in these types of cancer.

P049

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

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BACKGROUND-AIM

The prevalence of chronic rejection of the transplanted heart, also known as Cardiac Allograft Vasculopathy (CAV), increases with time after transplantation and is the leading cause of death of patients that survived after the first year. Cell free-DNA (cfDNA), released by dying cells has been proposed as an excellent biomarker of an ongoing rejection of the grafted organ, in several settings. Therefore, we reasoned that donor-heart derived cell free DNA (dd-cfDNA) could reflect the presence of an ongoing subclinical graft rejection and may be useful to monitor graft status in apparently stable transplanted patients.

METHODS

We enrolled 99 patients that underwent HTx over the last 10 years; 53 patients had CAV, while 46 had not (no-CAV). In 51 patients, blood samples were analyzed by NGS to identify dd-cfDNA. Echocardiographic and coronary angiogram data were collected.

RESULTS

No clinical differences between CAV vs no-CAV patients were observed, aside from time from HTx (154.98 in CAV vs 96.62 months in no-CAV; $p < 0.0001$). Concerning laboratory data, dd-cfDNA fraction was significantly increased in CAV vs no-CAV (0,78% vs 0,14%; $p = 0.001$). We observed by univariate logistic regression that time from transplant, myocardial mass, deceleration time and dd-cfDNA were significant predictors of CAV occurrence. Importantly, dd-cfDNA remained significant at multivariate logistic regression, even when corrected for the other covariates.

CONCLUSIONS

dd-cfDNA is a strong predictor of CAV development, even when corrected for potential confounders. Moreover, the molecular profiling of other circulating nucleic acids may provide additional information mostly for the identification of new potential markers to predict disease evolution.

P050

P300-MEDIATED ACETYLATION IN MYC-TARGETED THERAPY IN CANCER

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BACKGROUND-AIM

Recently, the effects of histone deacetylase inhibitors (HDACi) in cancer were investigated and many HDACi are 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 tumor action is mediated by MYC oncogene.

Specifically, HDACi induce MYC acetylation in lysine 323 (MYCK323ac), resulting in MYC downregulation both at transcriptional and protein level, thus unlocking target gene expression, including TRAIL, and leading to cancer cell apoptosis.

MYC activates its target genes through the recruitment of the adapter protein TRRAP, which acts as a scaffold to bind complexes containing histone acetyltransferase (HAT), such as Tip60 and p400. This complex leads to a state of hyperacetylation activating target genes.

Here, we identified the HAT(s) responsible for SAHA-mediated MYC hyperacetylation targetable to block MYC oncogenic activity

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 treatment with SAHA and A485, alone and in combination and to evaluate p400, RUVBL1 and RUVBL2 after treatment with SAHA, A485 and Sorafenib alone and in combination. Immunoprecipitation experiments followed by mass spectrometry analysis was performed to evaluate the MYC interactome.

RESULTS

Western blot experiments performed in U937, a human AML cell line, treated with SAHA show an increase of p300 expression level associated to an increase of MYCK323ac signal, suggesting potential role of p300 in the epigenetic MYC regulation. 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 cells treated with SAHA and the p300 inhibitor, A485, alone and in co-treatment, confirm the involvement of p300 in MYC regulation. Moreover p400, RUVBL1 and RUVBL2, that are associated to p300, were 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 specific role of each partners in SAHA-mediated MYC regulation.

P051

MODULATORY EFFECT OF EXTRA VIRGIN OLIVE OIL POLYPHENOLS AND THEIR METABOLITES ON INFLAMMATORY RESPONSE IN INTESTINAL AND ENDOTHELIAL CELLS

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BACKGROUND-AIM

Bioactive compounds hydroxytyrosol (HT) and tyrosol (Tyr) coming from extra virgin olive oil (EVOO) have been shown to possess anti-inflammatory properties in vivo. It is well known that, once ingested with the diet, are largely metabolized in the intestine and liver, thus their sulphated and glucuronidated metabolites are the prevalent forms found in human plasma, urine and in the gut, reaching concentrations compatible with biological activity. The aim of this study was to evaluate HT and Tyr and their sulphated and glucuronidated metabolites the inflammatory response at intestinal and endothelial level against LPS and/or a hyperglycemia (HG) condition. Their modulatory action was assessed focusing on the alteration of tight junctions (TJ) proteins and the activation of cellular pathways, as MAPKs and NLRP3 inflammasome, which are found to be upregulated in chronic inflammatory diseases.

METHODS

The alteration of epithelial/endothelial barrier in Caco-2 and HUVEC cells monolayer, treated with LPS or HG alone or together with EVOO phenolic compounds and their metabolites, was evaluated through cell permeability tests (TEER, FITC-Dextran permeability assay) and through determination of the disruption and/or relocation of TJ proteins, in relation to redox-sensitive MAPKs modulation and activation of the NLRP3 inflammasome.

RESULTS

Obtained data showed that HG and physiopathologically relevant concentration of LPS increase cellular membrane permeability in both Caco-2 and HUVEC monolayers, through the alteration of TJ proteins, following the activation of pathways involved in the inflammatory process such as MAPKs and NLRP3 inflammasome. Contextually, the pretreatment with physiological concentration of HT, Tyr and their sulphated and glucuronidated derivatives induced a protective effect, limiting the alteration of TJ proteins and the activation of MAPKs and NLRP3 inflammasome.

CONCLUSIONS

All these findings suggest that EVOO-derived phenolics parental free forms and their major in vivo formed metabolites, which represent the largest part of a continually changing pool of compounds, are responsible, as a whole, for the observed beneficial effects in the prevention and amelioration of the major intestinal and cardiovascular degenerative diseases.

CIRCULATING EXTRACELLULAR VESICLES FROM OBESE WOMEN PROMOTE PRO-NEOPLASTIC FEATURES OF STROMAL FIBROBLASTS IN BREAST CANCER.

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BACKGROUND-AIM

Obesity strongly impacts breast cancer (BC) incidence, and prognosis. Studies indicated that the obese setting provides local and systemic modifications able to promote BC progression and influence tumor microenvironment, particularly involving stromal fibroblasts (SFs). The mechanisms behind this interplay are multifactorial and may involve important mediators, as adipokines and growth factors. Aside from them, extracellular vesicles (EVs), small membrane particles secreted by various cells, act as powerful regulators of cell-to-cell communication in BC. However, the impact of obesity-derived EVs in affecting tumor-stimulatory features of SFs was not properly addressed.

METHODS

Circulating EVs were isolated from the serum of normal weight [NW: body mass index (BMI)<24.9kg/m²], and overweight/obese (OW/Ob: BMI>25kg/m²) women, and characterized by Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA), and marker expression (Immunoblotting, IB). Experimental models were: immortalised human mammary control fibroblasts (CFs), the cancer-associated counterpart (exp-CAFs), human CAFs (hCAFs), and MCF-7 BC cells. SF phenotype was evaluated by proliferation, migration, invasion and contractility assays. Tumor-stroma crosstalk was assessed by co-cultures. Cytokine arrays dissected the potential player/s.

RESULTS

TEM and NTA showed oval or bowl-shaped EVs ranging between 50-200nm. The EV markers TSG101/CD81/CD63 were detected in EV fractions. Of note, OW/Ob women exhibited elevated serum EV levels. Treatment of CFs with OW/Ob-EVs resulted in increased motility, invasiveness, contractility and expression of CF markers. BC cells cocultured with conditioned medium (CM) derived from OW/Ob-EV-treated CF displayed higher growth and migration than BC cells cocultured with CM from NW-EV-treated CF. OW/Ob-EVs also increased pro-tumor activities of exp-CAFs and hCAFs. Finally, OW/Ob EV-treated CFs showed enhanced release of Fibroblast Growth Factor 19 and Cystatin C, both closely related to BC.

CONCLUSIONS

Our data shed light on the mechanisms by which EVs isolated from obese women may impact stroma-tumor crosstalk and drive BC progression. Because of obesity epidemic, this knowledge may hold great promise for future management of BC patients with obesity.

CHARACTERIZATION OF NEW RIPKS FAMILY MEMBERS: HIGHLIGHTS ON THE BIOLOGICAL ROLE OF RIPK2 AND RIPK4 IN CANCER

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BACKGROUND-AIM

Receptor-Interacting Protein Kinases (RIPKs) are a seven-member family of Ser/Thr protein kinases involved in host defense, inflammatory phenomena as well as in cell death. To date their role in tumorigenesis is still unclear. Little scientific evidence explains the direct involvement of RIPK2 and RIPK4 in pathogenesis, especially in cancer. Several cell death regulation strategies have been studied in order to improve anticancer therapies and, among these, the study of the RIP kinase family is particularly interesting, which offers a therapeutic alternative in cases of resistance to apoptotic processes. It is known that RIPK2, through its CARD functional domain, is able to trigger the activation of NF- κ B or the MAP kinase pathway, playing a fundamental role in the immune response and inflammation while RIPK4 appears to participate both physically and functionally in several TRAF-dependent pathways leading to the activation of NF- κ B pathway.

METHODS

Western blot, RT-qPCR, Immunoprecipitation, Transfection.

RESULTS

Since both kinases are differentially expressed in different cancer types and the molecular mechanisms involved are poorly characterized, one of the main objectives of the project is to characterize the oncogenic role of these proteins by studying key molecular interactors and activated protein complexes. For this purpose, different cancer cell models exhibiting variable expression levels were chosen. Jurkat and Raji tumoral cell lines, in which RIPK2 is respectively poor and high expressed, as well as HL-60 and PANC-1 tumoral cell lines for RIPK4 investigation will be considered. To identify molecular players, MS/MS analysis coupled immunoprecipitation experiments were performed. Waiting for these results, molecular screening experiments have been started in order to identify molecules that modulate the expression of these kinases, that are structural scaffolds useful for the synthesis of new molecules.

CONCLUSIONS

Further studies will be needed to better understand the biological activity of the investigated proteins and to understand the possible cross talk between different family members.

ENIGMATIC ROLE OF RIPK1 IN LEUKEMIA

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BACKGROUND-AIM

Cell survival, inflammation and death are essential physiological events for maintaining cellular homeostasis and for preventing disease, especially cancer. These processes may be regulated by receptor-interacting protein kinase 1 (RIPK1), whose activity depends on post-translational modifications. While several advances in describing the molecular mechanisms involving RIPK1 are evident, the complex crosstalk in the regulation of its different functions in cancer makes it difficult to determine the precise events. Although the activity of RIPK1 in a wide range of diseases and in tumorigenesis has been demonstrated, its role in leukemia is still highly debated and therefore unclear. The heterogeneity of RIPK1 expression in leukemia patients and its enigmatic functions suggest to rapidly define the molecular mechanisms in hematologic malignancies.

METHODS

To study the degree of RIPK1 expression in several human leukemia cell lines we performed Western blot and qRT-PCR analyses. To gain further mechanistic insights, RIPK1 expression was evaluated in nutrient deficiency (% serum in medium). The proteasome inhibitor MG132 was used to define the molecular mechanism. More sensitive detection methods (Immunoprecipitation and biosensors) have been used to assess the relative amount of RIPK1.

RESULTS

RIPK1 protein in different leukemia cell models shows variable expression levels independent of its constant mRNA levels. Notably, despite the slight increase in PI positivity or alteration in cell cycle phases, U937 cells displayed divergent expression of RIPK1 protein after cell dilution and stress conditions. Indeed, nutrient deficiency down regulates RIPK1 which is restored after MG132 treatment. Furthermore, the more sensitive detection methods confirmed a reduced concentration of the target protein.

CONCLUSIONS

Our experiments confirm the variability of RIPK1 protein expression in leukemia and underline the hypothesis of a stress sensor whose mechanism depends on proteasome activity. Thus, these preliminary experiments are ideal for increasing our understanding of the transcriptomic and proteomic events underlying important biological processes that are not yet well characterized in leukemia.

PIRFENIDONE INHIBITS CANCER ACTIVATED FIBROBLASTS-CANCER CELL CROSS-TALK IN HUMAN NON-SMALL CELL LUNG CANCER CELLS

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BACKGROUND-AIM

Cancer-associated fibroblasts (CAFs) in solid tumors such as non-small lung cancer (NSCLC) are essential components of the tumor microenvironment and greatly contribute to the establishment of a fibrotic microenvironment that protects cancer cells from any blood-driven drug, favors cancer cell survival and epithelial-mesenchymal transition (EMT), and compromises the response to tyrosine kinase inhibitor therapy and immunotherapy. Pirfenidone (PFD) is an antifibrotic drug known to inhibit CAFs differentiation and the deposition of extracellular matrix (ECM) proteins. This study aims to investigate the effect of PFD in NSCLC behavior.

METHODS

A549 and H1975 NSCLC cells were exposed for 24h to the conditioned medium of CAFs. CAFs, isolated from human NSCLC patients, were in vitro exposed for 24h to PFD (1.5 mg/ml). The expression of different markers of EMT phenotype in NSCLC cells was evaluated in real-time PCR and western blotting analysis. The secretion of pro-matrix metalloproteinases (MMPs) and activated-MMPs was determined using gelatin zymography assays.

RESULTS

We found that: i) NSCLC cells exposed to PFD-CAF-CM express different mRNA profiles of EMT markers compared to cells exposed to CAF-CM; ii) the release of pro- and activated-MMPs was modulated in NSCLC cells exposed to PFD-CAF-CM; iii) the expression of Nrf2 transcription factor was inhibited in NSCLC cells exposed to PFD-CAF-CM.

CONCLUSIONS

It is known that PFD inhibits CAFs differentiation and cancer progression targeting TGF β signaling pathway on CAFs and cancer cells respectively. However, we suggest that, accordingly to our preliminary results, PFD perturbs cancer cells-CAF crosstalk exerting an additional potentiated anticancer effect targeting Nrf2 pathway. Indeed, Nrf2 expression represents a critical knot in lung cancer progression, since its constitutive and aberrant activation induces pro-survival genes and promotes cancer cell proliferation. Next, Nrf2 will be used as a target for anticancer treatment based on RNA technology in accordance with NextgenerationUE PNRR 2022 - CN 3 - National Center for Gene Therapy and Drugs based on RNA Technology - Spoke 2 - PhD program: The cross-talk between stroma and cancer cells in the tumor microenvironment as a target in therapies customized RNA.

UNRAVELLING THE HUMAN TYPE 3 DENDRITIC CELL MOUSE HOMOLOGUE: FROM SCRNAMEQ CROSS-SPECIES INTEGRATION TO AN ADENOCARCINOMA MOUSE MODEL.

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BACKGROUND-AIM

Conventional dendritic cells (cDCs) are innate immune cells that regulate inflammation and adaptive immune responses. In humans, cDC2s, a subset of cDCs, have been further classified into DC2s and DC3s. DC3s have been found to accumulate in chronic inflammatory conditions, including systemic lupus erythematosus, psoriatic skin, severe COVID-19, and advanced lung adenocarcinoma. Investigating the functions of DC3s in mice is crucial for in vivo studies. This study aims to identify and characterize DC3s in mice, validate their presence in normal tissues and tumors, investigate their spatial distribution within the tumor microenvironment, and explore their functional role in shaping T cell responses.

METHODS

- A cross-species integration analysis was conducted using a scRNAseq dataset comprising human and mouse lung cells to identify mouse DC3s.
- A flow cytometry-based method was established to distinguish murine cDC1s, DC2s, and DC3s.
- The established method was applied to normal tissues and tumors to confirm the enrichment of DC3s observed in advanced lung adenocarcinoma patients.
- Multiplexing analysis (30-plex) was employed to define the spatial distribution of immune system components within tumoral lungs.

RESULTS

- Mouse DC3s were successfully identified through the cross-species integration analysis.
- The flow cytometry-based method enabled the distinction of murine cDC1s, DC2s, and DC3s.
- Enrichment of DC3s was confirmed in normal tissues and tumors, consistent with observations in advanced lung adenocarcinoma patients.
- Spatial analysis revealed that DC2s were located outside the tumor area, while DC3s were found inside the tumor mass and in close proximity to T cells, including Tregs.

CONCLUSIONS

To further explore the role of DC3s within the tumor microenvironment, two techniques will be employed: cyclic immunofluorescence protocol and spatial transcriptomic/proteomic analysis using NanoString. These approaches offer single-cell resolution and will facilitate a comprehensive understanding of the functional contributions of DC3s. In addition, in vitro studies using sorted murine DC3s will be conducted to elucidate their impact on T cell responses. This study lays the groundwork for comprehending the functions of DC3s, their potential significance in tumors, and their potential as promising targets for future therapeutic interventions.

P057

ASTROCYTE SENESCENCE AS PATHOGENETIC MECHANISM UNDERLYING SEX DIFFERENCES IN ALZHEIMER'S DISEASE ONSET

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BACKGROUND-AIM

Alzheimer's disease (AD) is one of the principal neurodegenerative disorders whose incidence increases with advancing age and disproportionately affects women. Cellular senescence is a central hallmark of aging and age-related diseases, and since astrocytes represent a major cell population in CNS, senescent astrocytes may contribute to neurodegeneration. To study astrocyte senescence in AD, we treated human primary astrocytes from healthy subjects and AD patients of both sexes with A β 1-42 (A β). The first aim was to analyze if cells from AD patients respond differently to A β treatment than those of healthy people. Second, we aimed to explore whether sex plays a role in susceptibility to senescence, which could potentially clarify the higher dementia prevalence in women.

METHODS

Cells were treated with A β in a medium with 2% FBS, and after 5 days, the senescent phenotype was evaluated by analyzing the expression of several senescence markers and senescence-associated secretory phenotype factors. The ability of A β to interact with the cells was evaluated by semi-quantitative analysis by confocal microscopy and the intracellular Ca²⁺ levels using Fluo-4-AM dye in flow cytometry. The cellular viability and apoptosis were detected by MTT assay and Annexin V/7AAD flow cytometry assay, respectively.

RESULTS

Our results showed that A β -induced senescence was significantly increased in AD patients' astrocytes but not in cells from healthy subjects. Indeed, AD astrocytes, especially those from females, showed significantly increased SA- β -gal activity (30.4% females and 19.2% males) and the expression of pH2AX and macroH2A.1 foci, and p14ARF. Additionally, astrocytes of AD patients showed increased IL-6 expression, and only AD females showed increased IL-8 levels. This phenomenon was not observed in astrocytes from healthy people. Moreover, A β interactions in both cells of AD and healthy subjects increased intracellular Ca²⁺ levels. This increase seemed to correlate with an augment in the percentage of apoptotic cells in healthy subjects compared to AD patients.

CONCLUSIONS

During our study, we discovered that A β could cause senescence in cells of AD patients, especially in females, unlike healthy subjects who experience apoptosis instead. This was likely due to a rise in intracellular Ca²⁺ levels. The induced senescence in astrocytes from AD patients, especially in females, could contribute to neuroinflammation, leading to the progression and worsening of the disease in women.

ASPARAGINE TRANSPORT IS A METABOLIC TARGET FOR BCP-ACUTE LYMPHOBLASTIC LEUKEMIA THERAPY

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BACKGROUND-AIM

In most B-cell-precursor Acute Lymphoblastic Leukemia (BCP-ALL) patients, leukemic blasts have a low expression of Asparagine Synthetase (ASNS) and are dependent on extracellular asparagine (Asn), providing a rationale for the treatment with L-asparaginase (ASNase). In ALL niche, mesenchymal stromal cells (ALL-MSCs) are driven to supply Asn to the blasts, favouring resistance to ASNase. Asn transport is, therefore, of pivotal importance for blast survival, but the carriers involved have not been identified yet.

METHODS

Human ALL cell lines (RS4;11, REH, NALM-6, 697) were treated with natural or synthetic competitors of putative Asn carriers: Threonine (Thr, 10mM) or V-9302 (10μM) for ASCT2; Histidine (His, 10mM) or Glu₇HA (1mM) for SNAT5. Intracellular Asn levels were measured through LC-MS. ALL cell death was evaluated with cytofluorimetry in ALL monocultures or co-cultures with primary ALL-MSCs (n=3). Transporter expression and its correlation with minimum residual disease (MRD) at the end of induction therapy were evaluated in 71 paediatric ALL patients.

RESULTS

Among the tested lines, RS4;11 cells show the lowest ASNS expression and the highest sensitivity to ASNase, indicating that they fully depend on membrane transport for Asn supply. Intracellular Asn is significantly lowered by ASCT2 or SNAT5 silencing ($p < 0.05$) and by Thr and His ($p < 0.001$). When used together, the two amino acids markedly hinder ALL cell proliferation, while V-9302 and Glu₇HA inhibit mTOR, induce autophagy, cause a severe nutritional stress and a massive cell death. In contrast with the cytotoxic effect of ASNase, toxicity due to Asn transport inhibition is not prevented by co-culturing leukemic cells with ALL-MSCs. Moreover, V-9302 and Glu₇HA suppress the proliferation of the high-ASNS, ASNase-insensitive NALM-6 and 697 cells, but do not alter the viability of ALL-MSCs. ASCT2 and SNAT5 are consistently expressed in blasts of ALL patients at diagnosis, and ASCT2 expression is positively correlated with MRD ($p = 0.04$).

CONCLUSIONS

In conclusion, the survival of Asn-auxotroph ALL cells depends upon the Asn transporters ASCT2 and SNAT5, whose expression is associated with a lower response of ALL patients to induction therapy. Interference with Asn transport may thus represent a novel metabolic target for BCP-ALL treatment.

P059

ASSESSMENT OF GUT MICROBIOTA COMPOSITION IN HEALTHY PEOPLE OF FRIULI-VENEZIA GIULIA VIA NEXT GENERATION SEQUENCING (NGS)

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BACKGROUND-AIM

The gut microbiota constitutes a changeable ecosystem of symbiotic and commensal microorganisms with a fundamental role in metabolism, maintenance of intestinal mucosa, immuno-modulation and protection against pathogens. Gut microbiota constantly challenges by many variables, but, in healthy adults is mainly characterized by two major phyla: Firmicutes and Bacteroidetes. The composition of the human gut changes with age, ethnicity, lifestyle, dietary habits and geographic location. Given the growing role of microbiota as a modulator of physiological and pathological conditions, our study aims to investigate the genetic signature of the human gut microbiome in the Friuli-Venezia Giulia's healthy population.

METHODS

Bacterial DNA was extracted from stool samples using the QIAamp® PowerFecal® DNA Kit. Analysis of the V3-V4 hypervariable regions of bacterial 16S rRNA was conducted by Next Generation Sequencing (NGS), using the MiSeq system (Illumina). Data were computed, aligned, and grouped into operational taxonomic units (OTUs). The relative abundance of phylum, class, order, family, and species was defined after a normalization for the 16S copy number.

RESULTS

We characterized the gut microbiota of 109 healthy subjects. Data obtained allowed to define the normality range of phyla, classes, orders, families, genera, and species, both as absolute terms and as relative abundance. Firmicutes was the most represented phylum (51.1%), followed by Bacteroidetes 38.3% and Actinobacteria 3%. Considering classes, Clostridia 45.2% and Bacteroidia 37.7% were highlighted, while as regards of order Clostridiales 46.9%, Bacteroidales 26.6% and Anaeroplasmatales 12.6%. Finally, the most frequent families were Lachnospiraceae (21.9%) and Ruminococcaceae (16.2%), while *Faecalibacterium_prausnitzii* (10.3%), *Bacteroides Bacteroides_vulgatus* (4.6%) and *dorei* (3.5%) the most recurrent species. For each subject, the alpha-biodiversity, the enterotype, the obesity ratio, the gram-positive/gram-negative ratio and the analysis of individual taxa were classified.

CONCLUSIONS

This study represents a step forward in our knowledge of microbiota communities in Friuli Venezia Giulia. Data obtained provided information regarding the "baseline gut microbiota" and helped us drawing conclusions about how changes in the surrounding environment induce community-wide alterations in gene expression. Further investigations are needed to characterize the gut microbiota in dysbiosis states such as infectious diseases and antibiotic resistance (AMR).

P060

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 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 cancer, 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.

P061

CELLULAR CYBORGS LOADED WITH NEAR INFRARED GOLD NANOPARTICLES: A NEW APPROACH FOR PANCREATIC MALIGNANCIES (CYBERNATIC)

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BACKGROUND-AIM

Pancreatic cancer (PC) is a highly lethal disease with the lowest patient survival rate of any solid tumor. The failure of tumor response to the current available chemo- and radiotherapy may be related to emerging data demonstrating the importance of the tumor microenvironment in regulating therapeutic efficacy. The application of nanomaterials in the fields of medicine and biotechnology is of enormous interest, particularly in the areas where traditional solutions have failed. Nanoparticles can offer clear advantages compared to traditional therapies. 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

The present work describes the effects and the biological interactions of ECFCs loaded with AuNRs on 2D and 3D Pancreatic adenocarcinoma cell cultures (BxPC3 and PANC-1). Conventional optical and Transmission electron microscopes (TEM), Photoacoustic imaging (PA) and Inductively Coupled Plasma (ICP) were used to evaluate AuNRs intracellular uptake in ECFCs. Clonogenic assay, western blot analysis, real time PCR together with migration assay were employed to investigate the behavior of AuNRs-ECFC in co-culture with BxPC3 and PANC-1.

RESULTS

The PA signal provided from ECFC loaded with AuNRs exhibited a stronger enhancement thus confirming their role as "optimal cargo cells" for delivering AuNRs to the tumor. Indeed, AuNRs -loaded ECFCs enhanced AuNRs optical properties and exhibited an excellent thermotransductive property when exposed to Near infrared light (NIR). ECFCs retained their tumor tropic property and their ability to migrate towards tumor cells was even enhanced when loaded with AuNRs.

Moreover, ECFCs exert their antitumor activity by reducing the number of the colonies and modulating the epithelial mesenchymal transition (EMT).

CONCLUSIONS

We demonstrated in vitro that AuNRs-loaded ECFCs are able to generate a very high photoacoustic signal. Co-cultures of adenocarcinoma pancreatic cell lines with ECFCs showed an antitumor activity of ECFCs per se, enhanced by the presence of AuNRs by decreasing the number of the colonies. 3D culture spheroids confirm the antitumoral effect of AuNRs-ECFC. We also observe an enhanced long-term effect of nano-mediated exposure to Near infrared Light.

BPIFB4 AS A PROGNOSTIC TOOL FOR HUMAN FRAILTY

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BACKGROUND-AIM

Frailty is a state of increased vulnerability to stressors arising from the systemic decline in physiological reserve mechanisms with aging. Frail older people are characterized by five main components as weight loss, weakness, self-reported exhaustion, slow gait speed and low physical activity. Advanced age impacts on frequency and phenotype of immune cells. In this context, BPIFB4, a host defense protein with an immunomodulatory activity, has been found to be protective in healthy long living individuals in whom monocytes and macrophages have a favorable redistribution and phenotype. Although we previously found an inverse correlation of the homozygous LAV-BPIFB4 haplotype with frailty in elderly subjects, to date the role of the circulating BPIFB4 levels as frailty biomarker has not been characterized. Thus, the aim of this study is to investigate the correlation between BPIFB4 levels in recruited frail subjects and both their frailty assessment/health status and mono-macrophage phenotype.

METHODS

In this study, a group of 40 individuals (mean age 75 years; n=14 female, n=26 male) was recruited. Participants were subjected to standardized questionnaires to assess frailty risk, routine clinical examinations and blood test, monocytes and macrophages extraction with next immunophenotypic FACS analysis (through the FACS analysis of CD14, CD16, CD86 and CD163 markers).

RESULTS

Overall, 70% of the cohort has mild frailty, 25.5% has moderate frailty, and 5% has severe frailty. Compared to healthy controls, frail subjects show lower levels of circulating BPIFB4 that inversely correlate with the IRR for hypertension and cardiovascular disease. Flow cytometry results indicate total circulating monocyte frequency is reduced in frail subjects as compared to healthy controls. Considering monocytes' subsets, CD14⁺⁺CD16⁻ classical monocytes and non-classical CD14⁺CD16⁺⁺ monocytes are significantly increased in frail people compared to old controls, whereas intermediate CD14⁺⁺CD16⁺ monocytes are reduced. Moreover, also the M2/M1 macrophage balance is altered in frailty condition compared to old volunteers. No relationship between BPIFB4 plasma levels and monocytes' subsets was found.

CONCLUSIONS

Our findings highlight BPIFB4 protein has a potential prognostic value for marking the frailty condition.

P063

ANTIFIBROTIC DRUG PIRFENIDONE SENSITIZES HUMAN MELANOMA CELLS TO DOXORUBICIN: A POSSIBLE MECHANISM OF ACTION

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BACKGROUND-AIM

Pirfenidone (PFD) is a pyridone derivative, that has been authorized for the treatment of Idiopathic Pulmonary Fibrosis (IPF) in Europe, the United States, and other countries. PFD exerts its antifibrotic effect through the reduction of transforming growth factor β 1 (TGF β 1), and its anti-inflammatory property depends on the inhibition of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α). PFD exerts its anti-fibrotic and anti-oxidant activity by scavenging hydroxyl radicals and superoxide anions. Recently, PFD has been proposed in Phase I/Ib trial in combination with standard first-line chemotherapy for non-small cell lung cancer (NSCLC) treatment. Besides the inhibition of the TGF- β 1 pathway, the precise mechanism of action of PFD are still under investigation. Here we suggest a possible mechanism of action in a preclinical model of melanoma.

METHODS

M21 human melanoma cells were treated with PFD alone or in combination with sublethal doses of the antineoplastic drug doxorubicin (DOXO). PFD was administered accordingly to the recommended oral dosage in a range between 0,5 and 1,5 mg/ml. Cell viability was determined using MTT assay, AnnexinV/PI cytofluorimetric assay, and western blotting assay. The expression of matrix metalloproteases was determined using a gelatin zymography assay. Invasiveness was determined using Geltrex-coated transwells, and migration using the wound healing assay. Radical oxygen species were determined using MitoSOX cytofluorimetric assay.

RESULTS

We found that high doses of PFD induced the arrest of melanoma cell proliferation through the induction of necrosis and enhance radical oxygen species. Moreover, PFD potentiates the cytotoxic effect of DOXO. In addition, low doses of PFD induced the inhibition of melanoma cell migration and invasiveness.

CONCLUSIONS

The recommended high doses of PFD, in IPF patients, increase the risk of idiosyncratic toxicity that is mediated by reactive electrophilic intermediates generated by cytochrome metabolic activation. We suggest that in melanoma cells highly expressed cytochromes may support the generation of electrophilic species that 1) react with cellular protein, such as cellular glutathione (GSH), enhancing radical oxygen species, 2) lead to protein covalent binding that enhances cytotoxicity.

P064

A NEW IN VITRO MODEL OF BBB AND BRAIN ORGANIDS TO STUDY THE ROLE OF MG IN BRAIN DEVELOPMENT

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BACKGROUND-AIM

Human brain organoids are the widely used in vitro model that contribute to our knowledge about the biology and pathophysiology of nervous cells. They are 3D multicellular clusters that mimic the cytoarchitecture and the developmental pathways that occur in vivo. The most debated limitation of this in vitro model is the lack of the endothelial component. Thus, we have developed a co-culture in vitro system composed by the Blood Brain Barrier (BBB) and human brain organoids (ORGs). To investigate the strength of this in vitro model, the structure of the ORGs was assessed by evaluating the cortical layer organization. The modulation of BDNF, the crucial neurotrophic factor involved in neurodevelopment, neuroplasticity and neurosurvival, was observed. Moreover, we evaluate the role of magnesium (Mg) in neurodevelopment.

METHODS

ORGs are generated from iPSCs cultured with different differentiation media for 36 days. The BBB model is composed by a co-culture of human brain endothelial cells and human astrocytes in a transwell system. An inorganic and an organic Mg salt (Mg sulphate and Mg pidolate, respectively) were added to the culture media to reach the extracellular concentrations of 1 or 5 mM. The cortical layer differentiation was observed by immunofluorescence using CTIP2, TBR2 and SOX2 antibodies to detect the cortical, the subcortical neurons and the neural progenitor cells, respectively. Ultrastructural analysis was performed using light and transmission electron microscopy. BDNF was detected by ELISA.

RESULTS

Our data demonstrate that BDNF levels are higher in the new in vitro model mainly because of the presence of the endothelial component which is the main responsible for its secretion. Moreover, the cortical layer is more organized in the presence of the BBB. In addition, high Mg salts concentration (5 mM) ameliorates the organization of the ORGs cultured in the presence of BBB.

CONCLUSIONS

This study underlines the importance of the cross-talk between BBB and brain and unveils the role of Mg in brain development and in modulating the release of BDNF from the BBB.

RETINAL AND CIRCULATING MIRNA EXPRESSION IN PATIENTS AFFECTED BY VITREORETINOPATHY

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BACKGROUND-AIM

Inflammation and oxidative stress are important parameters in the pathophysiology of the major ocular diseases, mediated by NFκB, responsible from inflammation to apoptosis and Nrf2 with antioxidant as well as anti-inflammatory properties. Biological fluids such as serum and humor vitreous, are of great interest both as molecular targets for understanding molecular mechanisms that are not still well define in ocular diseases. The expression of miRNA may regulated protein-coding genes, suggesting their key role in multiple biological processes. The aim of the study was to identify the differential expression of miRNAs in humor vitreous and serum of patients affected by vitreomacular disease (VMD) and rhegmatogenous retinal detachment (RRD).

METHODS

Using the miRCURY LNAtm miRNA Focus PCR Panel (Qiagen) the expression of over 150 miRs was assessed respectively on pooled serum samples and pooled humor vitreous samples from 19 VMD and n.19 RRD patients. VMD and RRD the sequences with a more consistent differential expression between vitreous and serum were selected and validated in each patient. Statistically significant difference between the two groups was evaluated by Student's t-test, and Spearman rank correlation analysis was applied to evaluate relationship between miR expression values and clinical parameters.

RESULTS

The analysis of the miRNA panels showed 105 commonly expressed sequences in the humor vitreous and in serum. The main significantly differences were observed for miR21-5p, miR451a, miR222-3p, miR144-3p, miR146a-5p, miR320a with higher fold change in humor vitreous. A significant negative correlation was detected between miR146, Superficial Capillary Plexus (SCP) and superior and foveal Deep Capillary Plexus (DCP) in RRD pathology. In VMD the upper SCP and DCP positively correlate with the miR146 and miR451 while central macular thickness parameter showed a positive correlation with miR320a, miR21 and miR222.

CONCLUSIONS

Our data demonstrate the upregulation of miR222, miR146a-5p, miR21-5p related to the regulation of inflammatory mediators through the NFκB pathway; miR21-5p, miR222, miR451a, miR320a related to angiogenesis and cell invasiveness and miR144, miR146a-5p to the regulation of the antioxidant activity through the NRF2 signaling, as visualized by the interactive network analysis tool miRTargetLink 2.0. Our study supports the possible use of humor vitreous for miRNA evaluation to understand the molecular mechanism involved in vitreoretinal diseases.

NEW STRATEGIES FOR CONTROL PROGRAMMED DEATH LIGAND-1 ENDOCYTOSIS TO IMPROVE CANCER CHECKPOINT INHIBITOR THERAPY

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BACKGROUND-AIM

Immune Checkpoint Inhibitors (ICIs) have dramatically changed the landscape of cancer therapy. Emerging evidences suggest that improved prognosis and clinical outcomes in melanoma patients subjected to anti-PDL-1 therapy is associated with high PD-L1 positivity. Tseng and collaborators showed that targeting PAI-1 through the PAI-1 inhibitor Tiplaxtinin (TPX) synergizes with anti-PD-L1 checkpoint blockade in a model of murine melanoma; PAI-1 induced the internalization of surface-expressed PD-L1, resulting in the reduction of surface PD-L1. Binding of PAI-1 to uPA/uPAR complex results in the recruitment of low-density lipoprotein receptor protein 1 (LRP1), which also mediates PD-L1 internalization, leading to a decrease of PD-L1 membrane expression and to a desensitization to PD-L1 inhibitors. Another limiting factor of ICIs is the PD-L1 packaging within extracellular vesicles, the so-called exosomal PD-L1. Recent studies have revealed exosomal PD-L1 as a mechanism of tumor immune escape. On these bases, we propose to inhibit PDL-1 endocytosis by uPAR/LRP1 complex blockade to maintain high-cell-surface levels of PD-L1 and to reduce the expression of exosomal PD-L1.

METHODS

A375M6 (metastatic melanoma) A549 (non-small cell lung cancer) and MDA-MB231 (breast cancer) were treated with TPX to evaluate the modulation of TPX treatment on membrane and exosomal PD-L1. Conditioned media from untreated and TPX treated cancer cells were used for exosomes isolation, through ultracentrifugation. Exosomes size distribution and particle concentration were characterized by Nanoparticle Tracking Analysis. PAI-1 and uPAR silencing by siRNA were performed to block PD-L1 endocytosis; uPAR/LRP1 antagonist peptides were synthesized to disrupt uPAR/LRP1 interaction. After treatment with siRNA or uPAR/LRP1 inhibitors surface and exosomal PD-L1 expression were evaluated by FACS, confocal microscopy and western blotting.

RESULTS

Our results evidenced that in cancer cell lines PAI-1 and uPAR silencing by siRNA or uPAR/LRP-1 complex inhibition are able to block the PD-L1 internalization, inducing an increase PD-L1 membrane levels. Moreover, we demonstrated that exosomes from cancer cells treated with TPX or uPAR antagonist peptides show a decrease of exosomal PD-L1 levels.

CONCLUSIONS

These finding demonstrated that uPAR silencing or uPAR/LRP-1 complex inhibition result in a significant increase in surface PD-L1 levels and in a decrease of exosomal PD-L1, opening the way for new combined therapeutic strategies with anti-PD-1/PD-L1.

BCR/ABL PROTEIN SUPPRESSION IN CHRONIC MYELOID LEUKEMIA: POSSIBLE ROLE OF LACTATE AND SIRTUIN1

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BACKGROUND-AIM

Chronic myeloid leukaemia (CML) is a stem cell-driven neoplasia characterized by the expression of the constitutively active oncogenic tyrosine kinase (TK) BCR/Abl. Incubation in low oxygen, a condition which characterizes the stem cell niches (SCN) of bone marrow, leads to BCR/Abl protein suppression. Consequently, leukaemia stem cells (LSC) residing within SCN are refractory to TK inhibitors (TKi) used for CML therapy, due to the lack of their molecular target. It is therefore important to deepen the mechanisms driving BCR/Abl suppression to design new strategies able to repress TKi-resistant LSC. Our previous studies showed that BCR/Abl suppression occurs when glucose approaches complete exhaustion. Here, we investigated the possible role of lactate in the regulation of BCR/Abl expression.

METHODS

K562 and KCL22 stabilized CML cell lines were cultured for 5/7 days in low oxygen atmosphere (0.1% O₂) cultures (LC1). Cells were treated with lactate and/or lactate transporter inhibitors (AR-C15585, Bindarit, Syrosingopine), a pan-Sirtuin inhibitor (MC2494) or a Sirt1 activator (SIR1720). At the end of LC1, BCR/Abl protein expression and intra-/extra-cellular lactate concentrations were determined. To estimate the maintenance of stem cell potential, cells were transferred to normoxic secondary cultures (LC2) and their repopulation capacity was measured.

RESULTS

Lactate addition to LC1 was found unable to act as glucose surrogate to prevent BCR/Abl protein suppression in low oxygen; lactate actually reduced BCR/Abl expression and delayed LC2 repopulation. On the other hand, lactate transporter inhibition in LC1 led to the maintenance of BCR/Abl expression/signaling and a faster LC2 repopulation. These LC1 showed high intracellular / low extracellular lactate concentrations and higher expression of the NAD-dependent histone deacetylase Sirt1. Sirtuin inhibition in LC1 reverted the effect of lactate transporter inhibitors on BCR/Abl expression and LC2 repopulation kinetics, while the treatment with Sirt1 activator prevented BCR/Abl suppression and led CML cells to rapidly repopulate LC2.

CONCLUSIONS

Our results establish a possible association between extracellular lactate accumulation and BCR/Abl protein suppression and suggest the involvement of Sirt1 in the lactate-modulated BCR/Abl expression, opening new chances for combined therapeutic strategies in CML.

EXPLOITING THE STING PATHWAY FOR THE THERAPY OF MYCN AMPLIFIED NEUROBLASTOMA

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BACKGROUND-AIM

MYCN amplification dramatically affects tumor behavior and associates with refractoriness to standard therapy in MYCN amplified neuroblastoma (MNA NB), 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) response and immune responses against tumor cells, by sensing cytosolic DNA derived from viral or endogenous sources (DNA damage).

Whilst MNA NBs are characterized by high levels of oncogene-derived DNA damage, they are typically associated with a 'cold' immunophenotype. Whether this might depend on STING pathway attenuation has remained so far unexplored.

METHODS

Transcriptomics, methylome and ChIP-Seq analyses, as well as treatments with 5-aza-2'-deoxycytidine (DAC) and DNMT inhibitors were performed to assess the epigenetic state of the STING pathway on primary tumors and/or a panel of non-MNA and MNA NB cell lines and MYCN-inducible models. The expression and activation of the STING pathway were evaluated by WB and qPCR. STING agonist (diABZI) and other inducers (olaparib+MK-8776) were used to assess its activity under different conditions.

RESULTS

The STING pathway is significantly less expressed in MNA compared to non-MNA NBs and cGAS and STING promoters are significantly more methylated in MNA NBs. Consistently cGAS and STING are undetectable in all MNA NB cells and STING pathway activation and I-IFN responses are functionally impaired in MYCN-driven cells. Inducible MYCN expression transcriptionally repressed STING, supporting the idea that MYCN directly enforces the suppression of this pathway. Mechanistically, cGAS/STING repression occurs through DNA methylation. Restoring cGAS/STING by transgene expression or by epigenetic derepression via DAC/DNMT inhibitors is sufficient to sensitize to STING pathway inducers in MNA NBs. The induction of I-IFN responses in these settings indicate that STING pathway reactivation might be sufficient to restore the secretion of pro-inflammatory cytokines in MNA NB.

CONCLUSIONS

These data reveal that the STING pathway is silenced in MNA NB through epigenetic mechanisms enforced by MYCN. Understanding and counteracting these mechanisms may provide new therapeutic opportunities for MNA NB based on STING pathway reactivation.

BIPHASIC CHANGES IN GLUTAMINE METABOLISM DURING LPS-DEPENDENT ACTIVATION OF HUMAN MACROPHAGESG. Taurino¹, M. Chiu¹, E. Griffini¹, O. Bussolati¹, M.G. Bainchi¹¹Laboratory of General Pathology, Dept. of Medicine and Surgery, University of Parma, Italy**BACKGROUND-AIM**

Endotoxin (LPS)-dependent macrophage activation promotes pro-inflammatory M1 polarization accompanied by metabolic reprogramming, characterized in the first phases by elevated aerobic glycolysis and a broken tricarboxylic acid cycle. However, at longer times of incubation, LPS induces Glutamine Synthetase (GS) expression, a change associated to M2 polarization. We recently found that in human monocyte-derived macrophages (MDM) food grade amorphous silica nanoparticles (ASNP) prevent LPS-dependent GS induction through the intracellular sequestration of TLR4 (Bianchi et al., *Nanomaterials* 2022 doi:10.3390/nano12132307). In this contribution we investigate how Gln metabolism changes during LPS treatment and the mechanism underlying ASNP-dependent suppression of GS induction.

METHODS

Human primary monocytes were isolated and differentiated to MDM (50 ng/ml M-CSF for 7 days) before being pre-exposed to 5 µg/cm² of ASNP. After 24h, cells were stimulated with LPS (1ng/ml) for further 24h. Gene expression was investigated by RT-PCR and, at protein level, by western blot or ELISA. Cell Gln and Glu content was determined by LC-MS/MS.

RESULTS

Gln/Glu ratio undergoes a clearly biphasic change during LPS treatment of MDM, with an early decrease followed by a late (24h) restoration. The time course of the secondary increase corresponds to the induction of GLUL, the gene that encodes for GS. Along with GS increase, LPS treatment also causes a late increase of the anti-inflammatory cytokine IL-10 and a massive induction of the Gln transporter SNAT5. All these changes are suppressed by pre-exposure to ASNP. However, when added to the culture medium, IL-10 stimulated the expression of GS even in MDM pre-exposed to ASNP.

CONCLUSIONS

These results indicates that: (1) long lasting LPS stimulation increases Gln concentration and induces an outward transport route, thus pointing to conditions permissive for Gln secretion by macrophages; (2) the lack of LPS-dependent GS induction in MDM pre-exposed to ASNP is likely attributable to the suppression of IL-10 secretion and confirm that ASNP can perturb the metabolic changes associated to human macrophage activation.

REGULATION OF THE HEDGEHOG PATHWAY BY MICRORNAS TARGETING THE ONCOSUPPRESSOR KCASH2

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BACKGROUND-AIM

KCASH2 is a negative regulator of the Hedgehog signaling pathway (Hh) which plays a crucial role in morphogenesis and tumorigenesis. KCASH2 promotes the proteasome-dependent degradation of the critical deacetylase HDAC1, blocking the activation of GLI1, the main transcription factor of the Hh pathway. We previously demonstrated that KCASH2 is downregulated in medulloblastoma samples, and in silico analysis suggest its involvement also in other tumor types. By an in-silico analysis, we have identified several putative miRNA binding sites on the 3' UTR region of *kcash2* gene. Then we have verified if these miRNAs may potentially modulate KCASH2 expression, and we have confirmed in vitro their negative action on the KCASH2 expression.

METHODS

The human KCASH2 3' UTR and putative miRNAs involved in regulation of KCASH2 were identified by the computational analysis. Hence, to characterize the role of the miRNAs in modulation of KCASH2 expression we performed the luciferase and WB assays.

RESULTS

Notably, we observed a significant decrease in the activity of a 3' UTR of KCASH2 upon the overexpression of miR-125b, let7f, mir-196b, and miR-24. Coherently, we observed that the presence of these miRNAs determines the reduction of KCASH2 protein levels. Overall, these findings suggest that mir-125b, let7f, mir-196b, and miR-24 are able to act as negative regulators of KCASH2 tumor suppressor.

CONCLUSIONS

The results shown led to the identification of mir-125b, let7f, mir-196b, and miR-24 as factors which are involved in the negative regulation of the KCASH2 tumor suppressor. The discovery of miRNAs as noninvasive circulating biomarkers may have a significant impact on prevention, early diagnosis, prognosis, and possibly the prediction of therapeutic response. Furthermore, the discovery of new ways to increase KCASH2 levels may lead to novel techniques for suppressing Hh signaling in Hh-dependent cancers, including MB.

P071

SALIVARY DNA IN THE FOLLOW-UP OF ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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BACKGROUND-AIM

Oral carcinomas are a subgroup of head and neck cancers, a heterogeneous group of tumors that can affect different sites of the oral mucosa. In particular, the most frequent histotype is oral squamous cell carcinoma (OSCC). Early diagnosis of primary, secondary, or relapsing OSCC is critical, and to date the diagnosis of recurrence is made on solely clinical bases, since no biomarkers are so far available. In this scenario, salivary liquid biopsy could be an invaluable and rapid diagnostic tool.

METHODS

Whole saliva from OSCC patients referred to the maxillofacial surgery department was collected prior and after surgery, at different time points, in dedicated collection tubes. Salivary DNA (sDNA) was extracted from whole saliva or from salivary exosomes, and quantified. Next Generation Sequencing was then performed with the Ion S5 GeneStudio System using the Ion 540 Kit-Chef and the Ion 540™ chip-kit (all ThermoFisher Scientific). Data were further confirmed using droplet digital PCR.

RESULTS

sDNA levels correlated with the pathological status of the patients, as clinical relapse is associated with increased quantity of sDNA. The mutations analysed are strongly associated with OSCC, and their frequency is the same in sDNA and in DNA contained within the exosomes. The frequency of mutations increases in circulating free DNA (cfDNA) that remains outside exosomes.

CONCLUSIONS

Tumor DNA is not selectively transported by salivary exosomes and is mainly found as circulating free DNA (cfDNA). Nevertheless, its analysis in whole saliva provides important information that can be widely exploited in the rapid diagnosis of primary, secondary, or relapsing OSCC.

P072

ORAL MICROBIOTA AS A NOVEL PREDICTIVE BIOMARKER OF RESPONSE TO IMMUNE CHECKPOINT INHIBITORS IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS

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BACKGROUND-AIM

Although immune checkpoint inhibitors (ICIs) have changed the treatment paradigm of non-small cell lung cancer (NSCLC), these drugs fail to elicit durable responses in the majority of patients. The gut microbiota, able to regulate immune responsiveness, is emerging as promising, modifiable target to improve ICIs response rates. Since the oral microbiome has demonstrated to be the primary source of bacterial microbiota in the lungs, we investigated its composition as potential predictive biomarker to identify and select NSCLC patients who could benefit of immunotherapy.

METHODS

To this purpose, 59 patients with stage IV squamous and non-squamous NSCLC candidates to ICIs, as monotherapy, were enrolled. Saliva samples were collected prior the start of treatment. Response to ICIs was assessed by CT with m.d.c. or PET/CT using RECIST criteria version 1.1 of 2009. Metagenomic analysis through next generation sequencing of the hypervariable region V1-V2-V3 of the bacterial 16S rRNA was performed using Microbiota solution A (Arrow Diagnostics) and analysis of the data were obtained working on online platforms.

RESULTS

NSCLC patients were dichotomized as "Responders" with a partial or complete response of at least 12 months, and "Non-Responders" who had disease progression before 12 months. A prevalence of the Burkholderiales order was found in Non-Responders ($p= 8.6 \times 10^{-4}$; False Discovery Rate (FDR) = 0.02). Moreover, higher levels of Comamonadaceae family (belonging to the Burkholderiales order) was observed in "Non Responders" compared to "Responders" ($p= 5.8 \times 10^{-5}$; FDR = 0.002). Finally, the significant association between Comamonadaceae abundance and poor prognosis was confirmed also at genus and species level (genus: $p= 7.7 \times 10^{-5}$, FDR = 0.005; species: $p= 2.4 \times 10^{-5}$, FDR = 0.007).

CONCLUSIONS

These data showed, for the first time, a significant association between oral microbiota and ICIs response in NSCLC patients. In particular, the higher prevalence of Comamonadaceae family in "Non-Responder" patients suggests its potential immunomodulatory role.

GLUTAMINE ADDICTION OF MULTIPLE MYELOMA SHAPES A PRO-TUMOUR BONE MARROW NICHE.E. Griffini¹, G. Taurino¹, M.G. Bianchi¹, C. Maccari¹, R. Andreoli¹, G. D'Amico², N. Giuliani¹, O. Bussolati¹, M. Chiu¹¹Dept. of Medicine and Surgery, University of Parma, Parma, Italy²Tettamanti Research Center, IRCCS Fondazione San Gerardo dei Tintori, Monza, Italy**BACKGROUND-AIM**

Multiple myeloma (MM) is a proliferation of malignant plasma cells in the bone marrow (BM), characterized by bone lesions and increased adiposity. MM is the only human cancer that is both glutamine (Gln)-addicted and Gln-auxotroph, and the BM of patients presents low-Gln/high-glutamate (Glu) levels compared to pre-malignant stages. We have recently demonstrated that this peculiar metabolic microenvironment impairs osteoblast (OB) differentiation of mesenchymal stromal cells (MSCs), thus favoring MM bone lesions. However, the effects on other BM cells, in particular on the oversized adipocyte population described in MM, remain to be characterized.

METHODS

Primary human BM MSCs and the human MM cell line RPMI8226 were grown in RPMI1640 supplemented with 4mM Gln and 10% FBS. MSCs were incubated in either adipogenic (0.5 mM 3-Isobutyl-1-methylxanthine, 5µM indomethacin, 50µM dexamethasone and 10mg/ml human insulin) or osteogenic medium (10⁻⁸ M dexamethasone and 50 µg/ml ascorbic acid) for 14-days. The uptake of ³H-Glu has been used to determine EAAT3 activity. Cell viability was evaluated by the resazurin assay.

RESULTS

Glu uptake was higher in undifferentiated MSCs than in OBs. Consistently, public transcriptional profiles of BM biopsies of healthy donors (n=7) or MM patients (n=16) revealed that the expression of the inward Glu transporter EAAT3 is higher in MSCs compared to OBs. In Gln-free conditions, MSCs produced and secreted Gln, a phenomenon boosted by extracellular Glu. In co-cultures, MSCs sustained MM cell growth by recycling MM-secreted Glu into Gln. This nutritional support was markedly impaired by either inhibition or silencing of Glutamine Synthetase and EAAT3 in MSCs, with a substantial decrease in MM cell viability. Lastly, in MSCs incubated under adipogenic conditions, Gln deprivation increased lipogenesis, assessed with Oil Red O staining and the expression of the adipocyte markers PPARG, LEP, and ADIPOQ.

CONCLUSIONS

These data point to a MM-driven metabolic pro-tumor BM niche in which MSCs feed Gln-addicted MM cells, and MSC differentiation is skewed from osteogenesis to adipogenesis. Several steps of these deranged pathways are amenable to pharmacological inhibition, pointing to possible novel therapeutic approaches to counteract MM growth or its effects on the BM niche.

P074

IMPACT OF AN ENGINEERED FUNCTIONALIZED SCAFFOLD IN PROMOTING CARDIAC TISSUE REGENERATION IN THE POST-MI RAT MODEL

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BACKGROUND-AIM

Cardiovascular diseases currently represent the leading cause of death worldwide. In this context, tissue-engineered acellular cardiac scaffolds are gaining attention as a potential solution to promote tissue protection and regeneration in the damaged heart caused by ischemia. INCIPIT, a European research project, aims to enhance engineered 3D functionalized patches that can attract stem/precursor cells, regulate left ventricular remodelling and support cardiac cell electrical coupling during the regenerative processes following a myocardial infarction (MI). Validating the biocompatibility of these patches and assessing their effectiveness in restoring cardiac functionality after MI are key aspects of this study. Moreover, due to the limited tissue regeneration rate of the myocardium, the recruitment of cells capable to re-build cardiac affected area is necessary.

METHODS

Citocompatibility of the scaffolds was verified with Propidium Iodide Flow Cytometry assays and cell migration ability was assessed through transwell migration assays. Expression levels of target genes were analysed with Real-Time PCR. In vivo study was conducted in four groups of rats: SHAM, SHAM + patch, ischemia/reperfusion, and ischemia/reperfusion + patch. Immunofluorescence analyses were conducted on the explanted hearts 28 days after patch implantation.

RESULTS

Migration assays conducted as part of the research demonstrated that our functionalized electroconductive patches have the capacity to attract mesenchymal stem cells and non-myocyte cardiac cells in rats. Gene expression analysis revealed the cardioinductive effect of patches on cardiac stem cells. Additionally, the performed in vivo studies provided further confirmation of the patches' capability to recruit stem cells within the damaged heart tissue and also emphasized their role in promoting the formation of new blood vessels.

CONCLUSIONS

Based on the obtained results, the innovative functionalized electroconductive patches have shown promise as a strategy for regenerating cardiac tissue. These findings provide valuable insights to support further in vivo studies in a large animal model and may lead to potential clinical trials in the future.

LABORATORY MANAGEMENT OF BILIARY COLIC AND JAUNDICE IN EMERGENCY DEPARTMENT

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BACKGROUND-AIM

Gallstones are hardened deposits (cholesterol, black or brown pigment stones) of digestive fluid formed in the gallbladder. Cholelithiasis could be asymptomatic unless there is an obstruction of cystic duct, bile ducts, or both. The most common symptom is abdominal pain; sometimes unspecific gastrointestinal symptoms may occur.

METHODS

A 29 yr man arrives to the ED with epigastric and abdominal pain. He refers prolonged intake of NSAID due to recent trauma. Laboratory tests highlight WBC 11770/uL, Hb 12.6g/dL, CRP 1.17 mg/L, GGT 156 UI/L, AST 93 UI/L, ALT 333 UI/L, total bilirubin 6.21 mg/dL, direct bilirubin 3.71 mg/dL; lipase, total and pancreatic amylase are normal. Ultrasound study shows a distended gallbladder with numerous intraluminal stones and no dilatation of bile ducts. Patient history reveals predominantly indirect hyperbilirubinemia and normal hepatic markers. Mild anemia with splenomegaly concludes for presumptive hemolytic genesis, not further studied. Subsequent investigations individuate reticulocytosis ($0.5381 \times 10^6/uL$), increased ferritin (684 ng/mL) and decreased haptoglobin (<10 mg/dL). The presence of thalassemia is escluded with HPLC phenotype and molecular NGS; G6PDH enzyme value is normal; RBC membrane disorder is investigated.

RESULTS

Peripheral blood smear reveals marked anisopoikilocytosis and the presence of spherocytes. NGS approach highlights heterozygosity of ANK1 (NM_000037.4): c.1814dup (p.Leu606PhefsTer15), a novel frameshift mutation not reported in literature yet. UGT1A1 gene promoter polymorphism results in UGT1A1*28 genotype, associated with a decrease in liver enzymatic activity and hyperbilirubinemia.

CONCLUSIONS

Spherocytosis is characterized by the presence of round, fragile RBC susceptible to premature destruction, due to mutations altering shape and membrane flexibility. Symptoms can range from mild or absent to severe. Typical clinical manifestations are anemia, jaundice, fatigue, dyspnea, splenomegaly and cholelithiasis, signs also associated with thalassemia. For this reason diagnosis relies on careful clinical evaluation, targeted blood tests and confirmatory genetic testing. Coinheritance of Gilbert syndrome increases the risk of gallstone development.

STANDARDIZING PTH: COMPARISON BETWEEN 2ND AND 3RD GENERATION PTH ASSAYS

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BACKGROUND-AIM

Currently, most laboratories use 2nd generation PTH immunoassays (IMA). They differ mainly in their antibody capture sites, therefore besides the entire biological and active form (1-84 PTH), they detect, in different proportions, also biologically inactive fragments which accumulate in patients with kidney disease as they have a long half-life (10'-40' versus 2'-4' of 1-84 PTH). The more recent 3rd generation method aims to overcome the discrepancies of previous generation IMA by using antibodies able to recognize only 1-84 PTH.

The aim of this study is to compare PTH results obtained from the 2nd and 3rd generation IMA in hemodialysis patients (HEP) and in healthy females (HF).

METHODS

PTH measurement is performed with both iPTH 2nd generation assay (Centaur XP/XPT, Siemens Healthineers) (2nd assay) and 3rd generation PTH assay (Liaison XL, DiaSorin) (3rd assay) in EDTA-K₂ plasma samples of 45 HEP (M:F=30:15; age=40-91 years) and of 48 HF (age=35-60 years).

RESULTS

In HEP, the correlation is very good (Spearman's rank correlation coefficient- $r_s=0.977$) although the 3rd assay constantly measures less than the 2nd one (2nd assay median=157.6 pg/ml versus 3rd assay median=66.6 pg/ml; Mann-Whitney test $p<0.05$; Passing Bablock regression $y=2.8+0.43x$). The degree of agreement of the two different IMA to correctly classify HEP on the basis of the National Kidney Foundation reference intervals (RI), is moderate (Cohen's $K=0.632$).

In HF, similarly to HEP, the correlation is very good ($r_s=0.972$); also in this case, an average underestimation by 3rd assay is observed (2nd assay median=59.9 pg/ml versus 3rd assay median=33.7 pg/ml, Mann-Whitney test $p<0.05$; Passing Bablock regression $y=4.11+0.48x$). According to manufacturer's RI, a good degree of concordance of the two IMA is found (Cohen's $K=0.733$).

Finally, the percentage distribution of PTH fragments between the two subject populations is evaluated [(2nd PTH - 3rd PTH)/2nd PTH]. As expected, in HEP it is definitely higher (over 50%) respect to HF (less than 50%).

CONCLUSIONS

The 3rd generation PTH assay seems more specific since only the active form of PTH was measured; this could contribute to harmonize discrepancies between different PTH IMA providing more definite information to clinicians.

P077

ROLE OF PATHOGENIC MICROVESICLES IN HEMOLYTIC UREMIC SYNDROME AND PROTECTIVE EFFECT OF THE DRUG NAB815

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BACKGROUND-AIM

Shiga toxin 2 (Stx2) associated to blood cell-derived microvesicles (MV) through Toll-like receptor 4 (TLR4) and globotriaosylceramide is the main pathogenic factor responsible for the transition from bloody diarrhea to life-threatening hemolytic uremic syndrome (HUS), a severe sequela of Shiga toxin-producing *Escherichia coli* (STEC) intestinal infections, presenting with hemolytic anemia, thrombocytopenia and acute renal failure. Currently, there are no effective therapies for STEC infection and, recently, we have identified and patented the molecule NAB815, a non-toxic derivative of the antibiotic polymyxin B, as inhibitor of the binding of Stx2 to TLR4 expressed by circulating cells.

METHODS

Pathogenic MV from human blood (80 ml) challenged with 2 nM Stx2 in the absence (Stx2 MV) and in the presence (Stx2-NAB815 MV) of the drug (0.01 µg/ml) were isolated, resuspended in 200 µl of PBS and characterized by capillary Western blotting (identification of their origin and of associated Stx2); finally, their toxicity was assessed on Vero cells (protein synthesis inhibition and cell death) and in the CD-1 mouse model.

RESULTS

Stx2 MV showed higher amount of the microvesicle marker Alix ($193.4\% \pm 25.8$, $p < 0.0005$), the leukocyte antigen CD45 ($144.6\% \pm 14.3$, $p < 0.01$) and the platelet antigen CD42a ($252.1\% \pm 125.3$, $p < 0.05$) compared to control MV and were toxic to Vero cells ($ID_{50} = 0.35 \mu\text{l}$, $p < 0.005$). The 7-day treatment of mice with MV Stx2 was non-lethal, while renal function was impaired after 3 days (blood urea nitrogen, 50.4 ± 4.7 mg/dl, $p < 0.01$). NAB815 was effective in reducing the formation of total (residual stimulation of $25.7\% \pm 24.3$, $p < 0.0005$), leukocyte-derived ($16.4\% \pm 27.4$, $p < 0.01$) and platelet-derived ($31.6\% \pm 36.7$, $p < 0.01$) MV containing Stx2 ($12.3\% \pm 21.3$, $p < 0.001$). Stx2-NAB815 MV showed reduced toxicity to Vero cells ($ID_{50} = 1.16 \mu\text{l}$, $p < 0.001$) and did not change renal function in mice (blood urea nitrogen, 37.0 ± 4.4 mg/dl, $p < 0.01$).

CONCLUSIONS

Since Stx2 associated to microvesicles via TLR4 was detected in the blood of patients the day before the development of HUS, administration of NAB815 in patients with STEC infection could be an innovative treatment for the prevention of HUS.

INVESTIGATION OF THE BIOLOGICAL CONSEQUENCES OF PROLONGED INHIBITION OF CELL CYCLE CHECKPOINT KINASES 1 AND 2 ON GENETIC INSTABILITY IN ACUTE LEUKEMIA CELLS.

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BACKGROUND-AIM

Several genes involved in DNA damage response (DDR) pathways are over-expressed in various tumors, thereby affecting the efficacy of chemotherapeutic agents. For this reason, DDR inhibitors have been developed to enhance the efficacy of DNA damage-based therapies. Although the preclinical and clinical results are encouraging, there is still some concern about the risk of increasing the genetic instability of the primary tumor generating secondary, more aggressive clones. Our aim was to evaluate the effect of long-term exposure to PF-0477736 (CHK1/2 inhibitor) on acute lymphoblastic leukemia (ALL) cell's genetic instability and to characterize resistant cells.

METHODS

We stabilized a resistant cell line (NALM-6R) by treating ALL cells, NALM-6, with increasing concentrations of PF-0477736 for up to one year. Resistant cells were characterized by chromosome binding analysis (CBA), FISH, SNP array, immunoblotting analysis and in vitro drug screening.

RESULTS

After one year of treatment, we increased the IC50 values of NALM-6 to PF-0477736 by a factor of 8 (8 #M). CBA defined the outgrowth of a novel major clone (95%) in NALM-6R, characterized by 45,X,-Y,t(5;12)(q33;p13),t(7;19)(q11;p13), while 5% of the cells belonged to the parental cells, 46,XY,t(5;12)(q33.2;p13.2). Although the overall copy number status was similar between the two cell lines, the SNP array analysis identified several alterations. In particular, three copy number loss regions containing 74 genes were detectable only in the parental cells. Among them, several genes involved in cell cycle (ATM and NPAT) and apoptosis (BIRC2, BIRC3, CASP1/5/12) regulation were detected. FISH and immunoblotting analysis confirmed the different copy number status of the ATM gene and the altered protein expression of the ATM-CHK2 pathway, respectively, between the cell lines. Finally, in vitro drug screening showed that NALM-6R cells were also significantly resistant to the chemotherapeutic agents doxorubicin and methotrexate compared to parental

CONCLUSIONS

Our results suggest that prolonged inhibition of CHK1/CHK2 kinases may increase the genetic instability of cancer cells. We also show that the expression of key genes of the DDR pathway can significantly modify the efficacy of DDR inhibitors and conventional chemotherapeutics in ALL cells.

EXTRAMEDULLARY HEMATOPOIETIC ALTERATIONS IN BETA-THALASSEMIA PARALLEL TUMOR-ASSOCIATED MYELOPOIESIS

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BACKGROUND-AIM

Beta-thalassemia (BT) patients have improved life expectancy due to better management of iron overload and transfusion-related infections, but cancer incidence in these patients has risen and is the second leading cause of mortality. Hematopoietic disorders in BT patients account for defects in stem cell quiescence, erythropoiesis and myelopoiesis with extramedullary hematopoiesis. We demonstrated that spontaneous breast cancers in transgenic mice (MMTV-NeuT, MMTV-PyMT) involve early transcriptional and phenotypic reprogramming of bone marrow (BM) hematopoiesis, producing an expansion of immature myeloid cells. These BM tumor-associated changes parallel increased extramedullary splenic hematopoiesis, a condition shared with BT hosts, which involves expansion of erythroid precursors and immature myeloid elements in the red pulp, causing partial regression of the white pulp.

METHODS

Immunophenotypic and transcriptional analyses were performed to gain insight into the shared and divergent molecular characteristics of deregulated splenic hematopoiesis in BT transgenic mice and tumor-bearing mice as controls.

RESULTS

Common traits between tumor-free BT (Hbbth3) transgenic mice and tumor-bearing mice, included an expanded splenic red pulp rich in MPO+, CD68+ or CD206+ myeloid subsets and IL4r+ myeloid-derived suppressor cells (MDSC). Macrophages in Hbbth3 spleen samples showed an iron accumulation pattern due to ineffective erythropoiesis and hemolysis. The WP contraction involved a decrease in Pax5+ B-cells in tumor-bearing mice and a reduction of T and B lymphocytes in Hbbth3 mice. Spatial transcriptomics analysis in spleens of WT, tumor-free Hbbth3 and tumor-bearing mice identified upregulated genes associated with myeloid differentiation, innate immune response, iron metabolism, and oxidative stress regulation. The transcriptional signature shared by granulocytic/monocytic cancer-associated MDSC was enriched in Hbbth3 and tumor-bearing mice, while WT genes are associated with antigen cross-presentation and T- and B-lymphocyte activation.

CONCLUSIONS

The shared features between extramedullary hematopoiesis of thalassemic and tumor-bearing hosts suggests that basal hematopoietic alterations could impact on the dynamics of tumor development in this peculiar hemoglobinopathy.

THYROID HORMONE DYSHOMEOSTASIS CONTRIBUTES TO THE PATHOGENESIS OF DIABETIC RETINOPATHY

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BACKGROUND-AIM

Thyroid hormones (TH) are well-known critical regulatory molecules with roles in vertebrate physiology and development, in particular acting as pivotal regulators of cell metabolism. The main hormones produced by the thyroid gland are tetraiodothyronine (T4) and triiodothyronine (T3). Although perturbation of TH signaling is associated with numerous diseases, its role in the pathogenesis of diabetic retinopathy (DR) has never been investigated.

METHODS

Experiments aimed to characterize the TH system in the retina have been conducted in db/db mice, as an in vivo model of type 2 diabetes, and in the human retinal Müller MIO-M1 cell line exposed to high glucose (HG), where regulatory processes have been highlighted.

RESULTS

In the db/db retinas, typical functional traits and molecular signatures of DR were paralleled by a tissue-restricted reduction of TH levels. In particular, a local low T3 state (LT3S) correlated with deiodinase 3 (DIO3) upregulation and DIO2 and TH receptors downregulation. Consistently, the expression of T3-responsive genes, including mitochondrial markers and microRNAs (miR133-3p, miR338-3p, and miR29c-3p), was reduced. In MIO-M1 cells, a feedback regulatory circuit was evidenced whereby miR133-3p triggered the post-transcriptional repression of DIO3 in a T3-dependent manner, while HG led to DIO3 upregulation through a nuclear factor erythroid 2-related factor 2 – hypoxia-inducible factor-1 pathway. Finally, exposing MIO-M1 cells to a culturing condition that recapitulates early DR phases, it was demonstrated that LT3S and HG determined the reduction of markers of both mitochondrial function and stress response, which was reverted by T3 replacement.

CONCLUSIONS

The obtained results suggest that, in the early phases of DR, a DIO3-driven LT3S may be protective against retinal stress. However, in the chronic phase of the disease, LT3S not only fails to limit HG-induced damage but also increases cell vulnerability may be due to persistent mitochondrial dysfunction.

P081

EFFECTS OF CEREBRAL SMALL VESSEL DISEASE (CSVD) PATIENTS' PLASMA ON BRAIN ENDOTHELIAL CELLS

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BACKGROUND-AIM

Cerebral small vessel disease (CSVD) is one of the most common types of cerebrovascular diseases. Even though the pathogenesis of CSVD is largely unknown, endothelial dysfunction has been suggested as the turning point in CSVD development. In this study we tested the effect of plasma from CSVD patients on human cerebral microvascular endothelial cells with the aim of describing the pattern of endothelial activation.

METHODS

Plasma samples from 3 groups of young subjects have been tested: group 1 consists of subjects referred to the neurological outpatient clinic of our Institution affected by CSVD but without history of cerebrovascular events (PTs: 8 F and 1 M; mean age 50.6 ± 7.7 years); group 2 involves control subjects without CSVD or other abnormalities at MRI scanning (CTRLs: 5 F and 2 M; mean age 51.0 ± 14.9 years); group 3 consists of healthy blood donors (BDs: 4 F and 2 M; mean age 33.3 ± 18.5 years).

Human Brain Endothelial Cells 5i (HBEC5i) were treated with 15% v/v of plasma for 24h and total RNA was extracted using the RNeasy Kits (QIAGEN).

RNAs were pooled prior sequencing to reduce gene expression-based variability and sequencing with TruSeq Stranded Total RNA with Ribo-Zero Human/ Mouse/Rat kit on HiSeq2500 (Illumina).

Statistical analysis was performed using GraphPad Prism Software v.5 (GraphPad Software).

RESULTS

After filtering low quantity reads, 219, 175 and 174 differentially expressed genes were highlighted comparing PTs, CTRLs and BDs with HBEC5i untreated cells, respectively (at \log_2 fold change ≥ 1.5). 115 transcripts were up-regulated and 104 were down-regulated in PTs-treated cells; 84 transcripts were up-regulated and 91 were down-regulated in CTRLs-treated cells; 87 transcripts were up-regulated and 87 were down-regulated in BD-treated cells. Differentially expressed genes were then subjected to pathway analysis and no significantly altered pathway was evaluated in BD related treatment. Regulation of p38 MAPK cascade (GO:1900744) was the solely pathway altered in CTRLs related treatment. Indeed, 36 diverse biological processes turned out to be deregulated after PTs treatment of HBEC5i among with the cytokine-mediated signaling pathway (GO:0019221).

CONCLUSIONS

Endothelial cells activate inflammatory pathways in response to stimuli from CSVD patients' plasma, suggesting the pathogenetic role of neuroinflammation from the early asymptomatic phases of cerebrovascular disease.

PARENCHYMAL RENAL CELL CARCINOMA (RCC) IS ASSOCIATED WITH OCCULT HEPATITIS B VIRUS INFECTION (OBI)

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BACKGROUND-AIM

Evidence exists of a significant association between chronic kidney disease and hepatitis B virus (HBV) infection, even in cases with occult HBV infection (OBI), which is the long-lasting persistence of HBV genomes in HBsAg-negative individuals. Furthermore, a higher prevalence of renal cell carcinoma (RCC) was reported in HBsAg-positive subjects, but no data are available on RCC in OBI subjects.

Aims of this study were (1) to evaluate the prevalence of OBI in patients with RCC and (2) to investigate the molecular virological features of HBV in tumour and non-tumour kidney tissues

METHODS

We prospectively enrolled all consecutive patients who underwent nephrectomy for malignant renal tumours at the Urology Unit of the Messina University Hospital from April 2019 to May 2021. In analogy, patients who received nephrectomy for kidney benign tumours were enrolled as control group (CG). Tumour and adjacent non-tumour tissues were collected at the end of surgery. Each specimen was divided in two parts, one for histology and the other immediately frozen and stored for molecular analyses. Serum samples were collected and stored at -80°C from each case. DNA extracted from the tissues and paired serum samples were tested for OBI by a very sensitive nested-PCR.

RESULTS

A total of 83 HBsAg-negative patients (54 RCC, 29 CG) were included in the study. All the patients had no clinical, biochemical or ecografic evidence of liver disease. OBI was detected in 19/54 (35.2%) RCC patients, in 1/29 (3.4%) CG patients (P=0.001), and in any of the sera collected from RCC and CG patients. Thirty-six/54 RCC cases had clear cell renal cell carcinoma (ccRCC) and 18/54 had papillary or chromophobe RCC. OBI was detected in 9/36 (25%) RCC and in 10/18 (55.5%) non-ccRCC cases (P=0.03). Further molecular analyses showed the presence of the replicative intermediate HBV cccDNA minichromosome in renal tissues from OBI cases, whereas no HBV DNA integration into the host genome was detected.

CONCLUSIONS

OBI is significantly associated with RCC, and particularly with the non-ccRCC histotype. The detection of replication competent HBV cccDNA both in tumour and non-tumour kidney tissues suggests that HBV may potentially replicate in renal cells.

P083

NOVEL INSIGHTS IN THE FUNCTIONAL INTERACTION BETWEEN ESR1 MUTANT BREAST CANCER CELLS AND FIBROBLASTS

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BACKGROUND-AIM

Although endocrine therapy (ET) has improved the clinical outcome of Estrogen Receptor Alpha (ER α)-positive breast cancer (BC) patients, resistance to these treatments occurs and leads to metastatic dissemination. Evidence showed that a reduced response to ET may be due to intrinsic factors, mainly those related to somatic mutations in the ligand-binding domain of ER α (ESR1 mutation), cooperating with extrinsic factors mainly related to tumor microenvironment components (e.g. fibroblasts). Thus, the aim of our study was to investigate the functional interaction between fibroblasts and ESR1 mutant cells, not yet fully elucidated.

METHODS

We performed co-culture experiments between MCF-7 BC cells expressing Y537S mutation knock-in by CRISPR/CAS9 and normal/cancer associated fibroblasts (NFs and CAFs) by collecting conditioned media (CM). We assessed several biological assays (trypan blue, soft agar assays, and boyden chamber transmigration assays) together with genomic and proteomic assays. Finally, we orthotopically co-implanted MCF-7 or MCF-7 Y537S with CAFs in xenograft models.

RESULTS

Our data showed how the exposure to BC cells derived CM induced an increase of proliferation and migration of both NFs and CAFs, although to a higher extent in the presence of mutant CM. On the other hand, we found that NFs and CAFs derived CM sustained the aggressive behavior of mutant clones in terms of proliferation and migration. Furthermore, transcriptomic analyses allowed us to identify Insulin-Like Growth Factor 1 Receptor (IGF-1R) as the most upregulated gene in mutant clones. Interestingly, also proteomic analyses converge to settling IGF-1R as a central hub in the direct interaction network of mutant cells. Thus, we tested a specific IGF-1R inhibitor (GSK1878305A) in both "in vitro" and "in vivo" studies as a potential agent interfering with the reciprocal stimulatory effects between fibroblasts and BC cells that sustain tumor growth and progression.

CONCLUSIONS

Our study indicates how a better understanding of the molecular mechanisms underlying fibroblast/BC cell interactions may provide novel potential targets (e.g. IGF-1R inhibitors) allowing personalized management of patients affected by BC patients, especially in those harboring ESR1 mutations.

P084

A NOTCH-MESSAGE IN A BOTTLE. EXTRACELLULAR VESICLES MEDIATE NOTCH SIGNALING AND A PROTUMORAL BEHAVIOR OF PRE-OSTEOBLASTS IN MULTIPLE MYELOMA.

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BACKGROUND-AIM

The bone microenvironment is crucial in multiple myeloma (MM) progression. Although mesenchymal stem cells support MM progression, differentiated osteoblasts inhibit MM cell proliferation and increase drug sensitivity. The oncogenic Notch signalling promotes MM cell proliferation, survival, and pathological interaction with the bone marrow microenvironment. We recently found that the Notch effect is also mediated by extracellular vesicles (EVs).

Since we found Notch signalling members in MM cell-derived EVs (MM-EVs) cargo, we aimed to assess if the presence of Notch signalling members in MM-EVs affects pre-osteoblast differentiation and the cancer-supportive activity. Moreover, we investigated if the modulation of Notch pathway in the EV-producing cells could modulate the cargo in terms of miRNAs related to osteoblastogenesis and their involvement in osteoblast differentiation.

METHODS

We silenced Notch2 and the ligands Jag1 and Jag2 by a lentiviral approach in the OPM2 MM cell line, isolated the MM-EVs, and used them to educate MC3T3E1 pre-osteoblasts differentiated or not. The cargo of MM-EVs was analysed for the miRNAs profile.

RESULTS

MM-EVs increase MC3T3E1 cell viability and hamper differentiation induced by ascorbic acid and beta-glycerophosphate assessed by qPCR of genes involved in osteoblastogenesis (Runx2, Col1a1, Alp, Oc) and through evaluation of calcium deposition. MM-EV effects are inhibited by the silencing of Notch2 and Jag1-2 in the EV-producing MM cells.

The analysis of MM-EV cargo showed an enrichment in miR505, which is involved in controlling osteoblastogenesis by repressing the Runx2 level. The silencing of the Notch pathway stimulates osteoblastogenesis by reducing miR505 in EVs cargo.

The anti-differentiative effect of Notch signalling members in EV-mediated communication with osteoblast progenitors is relevant since MM-EV mediated disruption of MC3T3E1 differentiation provides these cells with the ability to reduce OPM2 cell sensitivity to chemotherapy drugs (Bortezomib and Melphalan).

CONCLUSIONS

This is the first evidence that Notch members affect MM-EV ability to promote the pro-tumoral behaviour of the mesenchymal cells in the bone marrow microenvironment. Vesicular miR505 partially mediates this effect.

THERAPEUTIC POTENTIAL OF CO-SIGNALING RECEPTOR MODULATION IN HEPATITIS B

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BACKGROUND-AIM

Hepatitis B virus (HBV) is a non-cytopathic, hepatotropic DNA virus with the potential to cause a persistent infection, ultimately leading to life-threatening conditions such as cirrhosis and hepatocellular carcinoma. Since HBV infections can spontaneously resolve in immunocompetent adults, there is increasing consensus that deconvoluting the tolerogenic mechanisms driving the dysfunctional differentiation of adaptive immune cells within the liver should provide rational therapeutic approaches to eliminate chronic hepatitis B virus infection. However, the specific molecular targets for achieving this goal are not fully understood.

METHODS

In this study, we combined bulk, single-cell RNA sequencing (scRNAseq) and high-dimensional flow cytometry to characterize the co-signaling receptor repertoire induced upon HC-priming and the long-term fate of dysfunctional HBV-specific CD8⁺ T cells in mouse models of HBV pathogenesis.

RESULTS

Our findings demonstrate that early during intrahepatic priming, HBV-specific CD8⁺ T cells selectively upregulate co-inhibitory receptors PD-1, CTLA-4, and LAG-3, as well as co-stimulatory receptors OX-40, 4-1BB, and ICOS. While blocking the co-inhibitory receptors had no significant impact, activating 4-1BB and OX-40, but not ICOS, transformed these cells into potent antiviral effectors. Prolonged antigenic stimulation of intrahepatically-primed dysfunctional T cells led to the development of a self-renewing, long-lived, heterogeneous population with a unique transcriptional profile that only partially overlaps with other forms of T cell hyporesponsiveness. This population consists of a dysfunctional progenitor or stem-like (TSL) population and two distinct dysfunctional tissue-resident memory (TRM) cell populations, which are hierarchically related. While 4-1BB expression is maintained across all populations, OX-40 expression is limited to TSL cells. At later stages, only stimulation of 4-1BB, not OX-40, conferred antiviral activity to HBV-specific CD8⁺ T cells.

CONCLUSIONS

These findings highlight the therapeutic potential of co-signaling receptor modulation for chronic HBV infection and suggest that targeting all dysfunctional tissue-resident T cells, rather than solely the stem-like precursors, represents a promising strategy, distinguishing it from other conditions characterized by chronic antigenic stimulation.

HBV RE-INFECTION OR DE-NOVO INFECTION IN THE COURSE OF LIVER TRANSPLANTATION IN PATIENTS CHRONICALLY INFECTED WITH HBV, HBV/HDV, OR HCV

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BACKGROUND-AIM

Long-term anti-HBV prophylaxis has been highly effective in reducing the rate of HBV recurrence in HBsAg-positive patients or de novo infection in HBsAg-negative liver transplant recipients of anti-HBc positive hepatic grafts. However, HBV reinfection still occurs in several patients. Aim. To verify HBV reinfection in HBsAg-positive/HDV-negative and HBV/HDV coinfecting patients as well as de novo infection in HBsAg-negative liver transplant (LT) recipients during the LT procedures.

METHODS

Virological analyses were performed on sera and hepatic tissues (native liver, pre- and post-perfusion allograft biopsies) from 21 LT patients (10 HCV-positive/HBsAg-negative, 5 HBsAg positive and 6 HBV/HDV positive). Moreover, liver biopsies from 6/21 cases (2 HCV-positive, 2 HBsAg-positive and 2 HBV/HDV positive) obtained at 3 years post-LT were also analysed. Six of the 21 LT patients received anti-HBc positive liver graft.

RESULTS

At the end of surgery, 2/5 HBsAg-positive patients, 2/6 HBV/HDV co-infected patients, and 2/10 HCV-infected patients showed HBV DNA (range: 6×10^{-4} - 1×10^{-3} copies/cells) and HBV cccDNA (range: 2×10^{-5} - 2×10^{-3} copies/cell) in the liver. All these patients received anti-HBc positive liver graft. Moreover, liver biopsies from all these patients at 3 years post-LT were HBV DNA positive despite the persistent HBsAg negativity, showing the presence of occult HBV infections (OBI). Furthermore, tissues from HBV/HDV positive patients were also positive for HDV RNA, which was not detected at the end of LT. Sequencing analysis of HBV DNA isolated from native livers and allografts revealed that the viruses present in liver biopsies 3 years after LT were viral strains of the donors. Of note, an HBV positive LT patient showed HBV genotype D sequences in the native liver, whereas HBV genotype A was identified in the allograft both at the end of LT and 3 years after LT.

CONCLUSIONS

(1) OBI in anti-HBc positive liver graft may persist over time both in HBsAg-positive and HBsAg-negative recipients after LT; (2) HBV sequences that persist in the transplanted liver can be of donor origin; (3) In HBV/HDV positive recipients, HDV infects the transplanted liver after LT and the infection can persist for a long time.

CIRCADIAN METABOLIC AND IMMUNE REGULATION IN GASTROINTESTINAL INFECTIONS

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BACKGROUND-AIM

Circadian rhythm permeates all aspects of mammalian physiology, including the immune defense against infections. The rhythmic environment that a microbe finds inside its host, generated by daily fluctuations in the availability of resources and the nature of immune responses, is able to influence microbial virulence, level of colonization and damage to the host. In our recent study we demonstrated that the host response to the pulmonary infection against the opportunistic fungus *Aspergillus fumigatus* changes its magnitude and efficiency depending on the time of first contact with the microorganism. This differential response is driven by the circadian clock controlling many aspects of the immune response, including the catabolism of the essential amino acid tryptophan. Our research is now focused at elucidate in details the mechanism of circadian regulation of tryptophan metabolism and to establish whether this regulation modulates the immune response in the gastrointestinal tract.

METHODS

We resorted to different murine models of intestinal damage, including fungal intragastric infection with *Candida albicans* performed at different times of the day-night cycle, in which we assessed the degree of colonization, level of inflammation and immunological mechanisms involved. Moreover, we used epithelial Caco-2 cells grown as polarized monolayer as an in vitro model of human intestinal cells in which to study at the molecular level how the modulation of the circadian clock influences intestinal permeability and inflammatory response.

RESULTS

We obtained results confirming a circadian regulation of the tryptophan metabolism in the gastrointestinal tract. By genetically or pharmacologically modulating the circadian pathway and the tryptophan metabolic pathway, we have shed light on the molecular mechanisms of reciprocal regulation between the two systems. We demonstrated that such regulation contributes to the rhythmic host response to fungal gastrointestinal infections. Indeed, the circadian production of tryptophan metabolites contributes to promote immune suppressive mechanisms at specific times of the day, thus modulating the antimicrobial host immune response.

CONCLUSIONS

In conclusion, we demonstrated that the circadian rhythm at the intersection between metabolism and immune response underlies the diurnal changes in host-microbe interaction, thus paving the way for a circadian-based antimicrobial therapy.

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EXPLOITING AUTOPHAGY TO PREVENT FUNGAL OUTBREAK IN CLL PATIENTS IN THE ERA OF PRECISION MEDICINE

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BACKGROUND-AIM

Chronic lymphocytic leukaemia (CLL), the most frequent type of leukaemia in adults, is a lymphoproliferative disorder that is characterized by the expansion of monoclonal mature B cells in the peripheral blood, secondary lymphoid tissues and bone marrow. During the last years, relevant advances in the understanding of the biological mechanisms associated with CLL have led to the clinical use of small molecule kinase inhibitors (SMKI). The molecular targets of SMKI are often fundamental pathways involved in the orchestration of many signaling functions. Thus, the downstream interference in signaling cascades may cause significant and unanticipated immune disturbances. Among these, the dysregulated immune responses against fungi may predispose to opportunistic fungal infections with a high mortality rate. Despite the comparable immune dysfunction of drugs, not all patients are susceptible to fungal infections suggesting that additional risk factors are important. Defects in LC3-associated phagocytosis (LAP), a non-canonical form of autophagy, are considered an important risk factor that predispose to invasive fungal infections. Understanding the molecular, mechanistic, and dynamic processes at the intersection between autophagy and susceptibility to fungal infections upon treatment with SMKI in CLL may have significant medical implications on preventing fungal infections in susceptible patients.

METHODS

To evaluate whether targeted drugs may perturb the protective autophagy response against fungi, THP-1 human monocytes and RAW 264.7 macrophages were treated with venetoclax and ibrutinib, the two major SMKI used in CLL, and pulsed with *Aspergillus fumigatus*, one of the most common fungal pathogens in hematological patients. Cells were evaluated for the autophagy induction and inflammatory parameters. In parallel, PBMCs from CLL patients treated with venetoclax and ibrutinib have been isolated and evaluated as above.

RESULTS

As expected, we found that autophagy was modulated following SMKI treatment in the human cell lines and PBMCs from CLL patients. Notably, activation of LAP in CLL patients has been associated with the effect of targeted drugs on protective response to *Aspergillus*.

CONCLUSIONS

Together, our preliminary data suggest that studying in an integrated way the impact of autophagy and its modulation during targeted therapy not only may elucidate new mechanistic knowledge but could also offer novel therapeutic strategies to modulate autophagy and prevent fungal infections in humans.

INTEGRINS REGULATE HERG1 DYNAMICS: SIGNALING, MODELING AND ANTIBODY INTERFERING IN CANCER CELLS

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BACKGROUND-AIM

hERG1 channels are often aberrantly expressed in human cancers, where they regulate many stages of tumorigenesis. Because many of these processes strongly depend on regulated cell adhesion to the ECM, a unifying factor of these pleiotropic effects of hERG1 may be indeed the interaction with integrin receptors. hERG1 and the $\beta 1$ subunit of integrin receptors can form a macromolecular complex on the plasma membrane of cancer cells. We studied the dynamics of this complex formation.

METHODS

We performed electrophysiology, adhesion, immunofluorescence, flow cytometer and immunoprecipitation experiments using HEK-hERG1, HCT116 and PANC-1 cells. We built up a mathematical model. We have also applied a novel antibody in the format of a single chain diabody which is also able to disrupt the complex. The latter is part of a pool of antibodies which has been tested in vivo, through electrophysiological analysis, in human cardiomyocytes.

RESULTS

We unravel a novel signaling pathway by which the hERG1/ $\beta 1$ integrin interplay stimulates pro-migratory cell behavior. Integrin engagement activates girdin-dependent $G\alpha i 3$ proteins, and in turn Protein Kinase B (Akt). This promotes hERG1 translocation to the plasma membrane and the transient association with $\beta 1$ integrin. The hERG1/ $\beta 1$ integrin complex stimulates cortical f-actin dynamics and thus cell motility. We have also tested a bispecific antibody in the format of a single chain diabody, scDb. The latter was also tested for its cardiac safety on human cardiomyocytes showing null effect on the current. To interpret the slow biphasic kinetics of hERG1/ $\beta 1$ integrin complex formation and the associated membrane hyperpolarization, we developed a mathematical model based on a generic balanced-inactivation-like module.

CONCLUSIONS

Our findings delineate a novel mechanism, based on the hERG1- $\beta 1$ integrin interaction, whose dynamics shows biphasic behavior: integrin engagement stimulates hERG1 channel translocation to the plasma membrane, which increases IhERG1 amplitude and causes Vrest hyperpolarization; hERG1 assembles with the integrin, and remains in the plasma membrane in the closed conformation, which progressively restores the initial Vrest. The possibility to avail of a pool of antibodies in order to disrupt the complex, without cardiotoxic effects, will open the way for new pharmacological strategies.

JAGGED1, A NOVEL NON-CANONICAL PLAYER IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

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BACKGROUND-AIM

Jagged1 is a single-pass transmembrane protein belonging to the Delta-Serrate-Ligand (DSL) family that transactivates Notch receptors through a cell-cell contact. Recent works indicate that Jagged1 is processed by sequential proteolytic cleavages involving ADAM-17/TACE and PS/ γ -secretase complex, resulting in the release of Jagged1 intracellular domain (J1-ICD), which can translocate into the nucleus and favors the expression of genes correlated with proliferation and EMT, and decrease the apoptotic-related genes. Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant tumor characterized by almost universal constitutive activation of K-Ras (90% of cases). Interestingly, Jagged1 has been shown to be overexpressed in pancreatic cancer and associated with poor differentiation, large tumor size, metastasis, invasion and high TNM stage in PDAC patients. Furthermore, the oncogenic K-Ras controls ADAM17 activity through regulation of the MEK/Erk/ADAM17 signaling axis. Based on these observations, here we investigate the role of Jagged1 in PDAC.

METHODS

Several human PDAC cell lines were purchased from ATCC and cultured under standard conditions. PDAC cells silenced for K-Ras and/or Jagged1 were analyzed by in vitro and in vivo assays, in order to evaluate the role of Jagged1 in proliferative and metastatic events.

RESULTS

First, we show that Jagged1 is overexpressed in PDAC patients and that there is a positive correlation between K-Ras and Jagged1, which is associated with an unfavorable clinical outcome. We also show that Jagged1 is subject to constitutive proteolytic processing mediated by K-Ras signaling via regulation of the MEK/Erk/ADAM17 signaling axis and that its fragment, J1-ICD, is localized in the nucleus of several PDAC cell lines. By establishing overexpressing Jagged1 PDAC cell lines, we observe that J1-ICD presence correlates with a high proliferation rate, upregulation of EMT and anti-apoptotic related genes. On the contrary, in vivo experiments of xenotransplantation with PDAC cell lines Jagged1-depleted by CRISPR/Cas9 technology show an important reduction of tumor growth in xenograft derived by Jagged1-negative cells.

CONCLUSIONS

The preliminary data reported here suggest an intricate role of Jagged1 in PDAC initiation/progression, which must be further investigated. Therefore, our aim will be to discriminate the specific role of canonical and non-canonical Jagged1 in PDAC onset/development.

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EVALUATION OF FERRITIN LEVELS AND OTHER CIRCULATING PARAMETERS AS PREDICTIVE BIOMARKERS OF POSTOPERATIVE ATRIAL FIBRILLATION RISK

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BACKGROUND-AIM

Postoperative atrial fibrillation (POAF) represents a critical issue in the cardiovascular area as well as the most common arrhythmia post cardiothoracic surgery. POAF, thus, is associated with diverse severe complications, including potentially fatal conditions. The risk of developing stroke is particularly frequent in post-cardiothoracic surgery AF patients. Little is known about the pathophysiological mechanisms that lead to this fibrillation and there are currently no useful markers to predict it. Yet, it is known that ferritin, a protein that into the serum act as an iron carrier, it's correlated with an increased risk for atrial fibrillation.

METHODS

Based on this evidence, here, we evaluated, through a retrospective observational study, the validity of ferritin and other haematological parameters as POAF risk biomarkers in patients undergoing cardiac surgery. The cohort consists of 105 patients (mean age = 70.1 ± 7.1 years; 70 men and 35 females) with an history of cardiothoracic surgery. Haematological, electrocardiographic, and echocardiographic parameters were evaluated.

RESULTS

The data obtained evidenced that POAF patients showed significantly higher concentrations, absolute values, and percentages, of ferritin, RDW, and PLTs, respectively. However, after adjustment for other risk POAF variables, the ferritin resulted to be the independent factor associated with the onset POAF risk. Such result led us to identify the ferritin cut-off value, which, when equal to or greater than the value of 148.5 ng/mL, identifies the subjects with highest risk of developing POAF. These findings demonstrated, for the first time, the importance of detecting serum ferritin values, and if ≥148.5 ng/mL, the individuals subject to cardiothoracic surgery, this suggests a high probability of developing POAF after the surgery treatment in conventional extracorporeal circulation (CECC).

CONCLUSIONS

Thus, serum ferritin, RDW, and PLTs represent predictive biomarkers of POAF after cardiothoracic surgery in CECC; particularly, serum ferritin combined with anormal PW indices and structural heart disease variables can represent an excellent tool for predicting not only POAF, but also the eventual stroke onset.

STUDIES ON POTENTIALLY DRUGGABLE PATHWAYS THAT REGULATE STRESS-INDUCED TRKAIII MITOCHONDRIAL INTERNALIZATION, CLEAVAGE AND ACTIVATION IN HUMAN NEUROBLASTOMA CELLS.

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BACKGROUND-AIM

In aggressive pediatric neuroblastomas (NBs), neurotrophin receptor TrkA alternative splicing, resulting in TrkAIII variant expression, positively correlates with post-therapeutic relapse and advanced stage metastatic disease. In NB models, the TrkAIII variant promotes primary and metastatic tumorigenesis consistent with oncogenic function. Alternative TrkAIII splicing in NB cells is promoted by hypoxia, nutrient deprivation, DTT and A23187 in association with unfolded protein response (UPR) activation, implicating the tumour microenvironment in converting fully spliced TrkA tumour suppressing signals to TrkAIII oncogenic signals. UPR activation, induced by DTT and A23187, also promotes mitochondrial TrkAIII internalization, OMI-mediated TrkAIII cleavage and activation, resulting in PDK1-dependent glycolytic adaptation and enhanced stress-resistance. Here, we have investigated potential druggable pathways involved in this novel stress-induced mitochondrial protective mechanism.

METHODS

RT-PCR, mitochondria purification, regular and co-immuno-precipitation Western blotting, Incucyte cytotoxicity assays, and Student's t-test.

RESULTS

In TrkAIII expressing SH-SY5Y NB cells, DTT-induced mitochondrial TrkAIII internalization, cleavage and activation was associated with UPR activation, enhanced TrkAIII complexing with Grp78 and calmodulin, and was abrogated by Grp78 (HA15), calmodulin (W7) and Hsp90 (geldanamycin) inhibitors. DTT-induced intramitochondrial TrkAIII activation but not cleavage was also prevented by Trk (entrectinib), Ca²⁺ uniporter (DS16570511), TRAP-1 (Honokiol DCA) inhibitors and by the ROS scavenger Resveratrol. The clinically approved Trk inhibitor entrectinib significantly enhanced DTT-induced cytotoxicity in TrkAIII SH-SY5Y cells.

CONCLUSIONS

Under ER stress conditions, misfolded N-glycosylated TrkAIII exhibits Grp78, Ca²⁺ calmodulin and Hsp90-dependent mitochondrial internalization, where it is cleaved prior to being activated by a Ca²⁺ uniporter and oxidation-dependent mechanism, resulting in enhanced survival. This improved understanding adds and extends potential therapeutic inhibition of this novel pro-survival mechanism from Trk to GRP78, calmodulin, HSP90/TRAP1 and Ca²⁺ uniporter inhibitors.

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THE TRKAIII SPLICE VARIANT ONCOPROTEIN ENHANCES PD-L1 EXPRESSION IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS

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BACKGROUND-AIM

Neurotrophin receptor TrkA alternative splicing, resulting in TrkAIII variant expression, positively correlates with post-therapeutic relapse and advanced stage metastatic disease in pediatric neuroblastomas, MCPyV positive Merkel cell carcinomas and cutaneous malignant melanomas. The TrkAIII variant is characterized by exons 6 and 7 skipping, which encode regulatory domains, the absence of which result in intracellular accumulation, and ligand-independent cell cycle and stress-regulated activation. TrkAIII activation signals through PI3K-AKT but not Ras/MAPK, exhibits oncogenic activity in NB and melanoma models, and enhances both stress and chemotherapeutic resistance. Immune checkpoint inhibition mediated by programmed death ligand PD-L1 and its receptor PD-1 plays a significant role in NB pathogenesis but the regulation of this important immune-evasion pathway is poorly understood in NB. Here, we have investigated whether TrkAIII regulates PD-L1 expression and function in TrkAIII expressing SH-SY5Y NB cells.

METHODS

Real Time qPCR, RT-PCR and Western blot comparisons of PD-L1 mRNA and protein expression in control, TrkA and TrkAIII transfected SH-SY5Y cells, under untreated conditions and following treatment with entrectinib Trk, LY-294002 PI3K and PD98059 MEK inhibitors. PD-L1 function was assayed by IL-2 ELISA in co-cultures of control, TrkA and TrkAIII SH-SY5Y cells incubated for 48 hours with PHA and TPA-activated Jurkat T cells.

RESULTS

TrkAIII SH-SY5Y cells express significantly higher levels of PD-L1 than control or TrkA SH-SY5Y counterparts. Constitutive PD-L1 mRNA expression in TrkAIII but not control or TrkA SH-SY5Y cells was significantly inhibited by entrectinib and LY-294002 but not PD98059. Jurkat co-culture with control, TrkA and TrkAIII SH-SY5Y cells significantly reduced IL-2 production, confirming PD-L1 function.

CONCLUSIONS

The data implicate TrkAIII in promoting PD-L1 expression via PI3K in human SH-SY5Y NB cells, supporting a rationale for using clinically approved therapeutic Trk inhibitors in combination with PD1/PD-L1 inhibitors in TrkAIII expressing NB.

ERK5 NUCLEAR LOCALIZATION TARGETING SYNERGIZES WITH THE ERK5 KINASE INHIBITOR AX15836 IN REDUCING CANCER CELL PROLIFERATION

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BACKGROUND-AIM

The extracellular signal-regulated kinase 5 (ERK5) is emerging as a possible target for melanoma treatment. ERK5 pro-proliferative effects are linked to its presence in the nucleus. We focused on the elucidation of ERK5 nuclear translocation using single molecule tracking and searching compounds to prevent ERK5 nuclear shuttling, to design new strategies for cancer treatment.

METHODS

To achieve single ERK5 molecule tracking, we used super-resolution microscopy. HeLa cells were transfected with a vector for ERK5, linked to HaloTag, alone or with a vector for a constitutively active form of the ERK5 activator MEK5. The cell-permeable chromophore JaneliaFluor646 was used for the detection. As a complementary approach, HEK293T cells, transfected with ERK5 and MEK5DD, and A375 melanoma cells were treated with the α/β 1 importin-mediated transport inhibitor ivermectin (IVM) or transfected with two siRNA targeting importin- β 1. MTT, 2D-colony forming assays and apoptosis evaluation were performed in A375 or HeLa cells treated with IVM in combination with the ERK5-i AX-15836. A375 and HeLa spheroids have been used to evaluate the effect of IVM and AX-15836 on a 3D model.

RESULTS

The HaloTag technology provided the selective binding of JaneliaFluor646 to ERK5, and Highly Inclined and Laminated Optical sheet microscopy allowed to collect the signal of single ERK5 instances. 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 and this effect is partially reverted in cells treated with IVM. Moreover, ERK5 amount in the nuclear fraction of lysates from IVM treated-cells and from KPNB1 KD cells is lower compared to control, confirming a role of importin β 1 in ERK5 nuclear transport. Finally, we found that ERK5i AX-15836, which has been reported to induce paradoxical activation of ERK5 and its nuclear translocation, reduced melanoma cell proliferation only in combination with IVM in 2D and 3D growth models.

CONCLUSIONS

The present study demonstrated the involvement of importin α/β 1 in ERK5 nuclear translocation. Our data showed that impairment of ERK5 nuclear localization restores sensitivity to AX15836, suggesting that the actors involved in ERK5 nuclear shuttling could be exploited as novel targets for ERK5 inhibition, and therefore for additional anticancer therapies. The described super-resolution technique will also help future studies to investigate the mechanism of action of ERK5 in the nucleus.

STARTING POINT FOR EGFR THRESHOLDS REASSESSMENT ON THE BASIS OF AGE AND SEX/GENDER TO EASILY DIFFERENTIATE AGE-DEPENDENT RENAL FUNCTIONAL DECLINE FROM CHRONIC KIDNEY DISEASE (CKD) IN MEN/ WOMEN

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BACKGROUND-AIM

CKD in adult is defined by a value of estimated glomerular filtration rate (eGFR) less than 60mL/min/1.73m². The use of the fixed thresholds shows different caveats, e.g., CKD overdiagnosis in elderly people and the sex differences. Thus, age and gender differences should be considered.

METHODS

We evaluated the variability of eGFR in 57,449 adults divided in four age-classes (18-40, 40-60, 60-80,80-100) and considering the gender. Were also assessed the levels of leukocytes, inflammatory variables, and albumin/creatinine ratio, as well as neutrophils/lymphocytes ratio and the modified Glasgow prognostic score (mGPS). Statistical methodologies in use in our laboratory were also applied.

RESULTS

The data obtained showed that our eGFR has a significant negative correlation with age ($p < 0.001$), consequently to age's increases eGFR decreases. In addition, the two-way factorial ANOVA revealed the significant effects of Age Class ($p < 0.001$) and Gender ($p < 0.001$) on eGFR. By using a multinomial multivariate logistic regression model considering the stratification of the four age classes for eGFR categories, established by the fixed threshold: G1, G2, G3a, G3b, G4 e G5, we observed significant OR values for CKD. For example, the subjects aged between 18 and 40 years had the lowest OR values, with a decrease of a factor of 0.01 ($p < 0.001$), to develop G2-G5 pathological conditions than the oldest age-class (80-100 years). This < 1 risk trend is maintained in all eGFR strata, when compared with the oldest age-class. (80-100). In addition, to be female also reduced the risk to be G2-G5 with respect to G1 by a factor of 0.23 ($p < 0.001$). By analyzing the inflammatory risk according to the categories of mGPS, the subjects aged between 18 and 40 years showed the lowest OR values for mGPS2 than 80-100 years class people with a decrease by a factor of 0.03 ($p < 0.001$). This < 1 risk trend across age groups is maintained in all mGPS strata. To be female also appeared significantly to decrease the risk to develop of a factor of 0.44 ($p = 0.009$), given that the other variables in the model were held constant. This < 1 risk trend for Gender is maintained in all mGPS strata.

CONCLUSIONS

This study proposes modifying the current eGFR values for CKD across the age groups and by sex/gender.

MONITORING SARS-COV-2 OMICRON VARIANT EVOLUTION IN PATIENTS TREATED WITH SOTROVIMAB

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BACKGROUND-AIM

Sotrovimab is a monoclonal antibody used as monotherapy in patients affected by COVID-19. Among patients with the delta variant and treated with Sotrovimab, 4% rapidly developed resistant mutations in the spike protein at positions 337 or 340. Genomic surveillance by Next Generation Sequencing (NGS) has been applied since the beginning of the COVID-19 pandemic to track the spread of the virus. Furthermore, using saliva as specimen collection facilitates the implementation of a less invasive sample during patients follow up. In this study, we applied a combinatory strategy of saliva collection and NGS for SARS-CoV-2 mutation detection in patients treated with sotrovimab.

METHODS

In this observational study, 43 patients were enrolled. Saliva samples were collected at the beginning of the therapy with Sotrovimab. Viral RNA was extracted using the Qiasymphony instrument. Libraries for NGS analysis were prepared according to the Illumina protocol. A clinical database containing patients' demographics was constantly updated. Statistics were performed with STATA6 software and significance was set at $p=0.05$.

RESULTS

NGS allowed us to characterize SARS-CoV-2 variants in all 43 patients enrolled. Among patients treated with sotrovimab, the BA.1 lineage was found in 55.8%, while the BA.2 like in 44.2%.

The resistance mutations on amino acid 337 were developed in 4,6% of patients: 2.3% showed the P337H substitution while the other 2.3% was the P337S. Considering clinical correlation, the association between fever onset and spike mutations emerged. Significant 4 mutations were associated with fever: the N440K, (OR: 8.75, CI: 1.42-53.91, p -value: 0.019), the N510Y (OR: 5.63, CI: 1.10-28.8, p -value: 0.038), the Q493R (OR: 5.63, CI: 1.10-28.84, p -value: 0.038) and Q498R (OR: 5.63, CI: 1.10-28.84, p -value: 0.038).

CONCLUSIONS

We detected resistance mutations in the spike protein in all patients tested. The specific substitutions at positions 337, was found in 4,6% patients with omicron lineage treated with sotrovimab. Notably, these mutations have been exclusively reported after sotrovimab treatment in immunocompromised patients. As previously reported, the monoclonal antibody as monotherapy in patients is a risk for escape mutant selection that might hamper viral clearance. Patients treated with monoclonal antibodies should benefit from a virological follow-up, including viral sequencing and viral load assessment based on saliva samples to monitor the virus evolution.

REGULATION OF ARGININE METABOLISM IN MACROPHAGES HOST CELLS BY LEISHMANIA INFANTUM AND HYPOXIA

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BACKGROUND-AIM

Leishmaniasis is a neglected tropical disease caused by the obligate intracellular protozoa *Leishmania*. In hypoxic and inflamed infected tissues macrophages, the main host cells for parasite replication, contribute to the disease outcome through the regulation of arginine metabolism. Arginine is the common substrate of inducible nitric oxide synthase, producing the microbicidal nitric oxide (NO), and of the two isoforms arginase 1 and arginase 2, promoting *Leishmania* survival through polyamines production. How *Leishmania* infection and hypoxia regulate the metabolism of arginine in macrophages is not completely known, thus the aim is to study the metabolism of arginine in an in vitro model of *Leishmania* infected macrophages in normoxia or hypoxia.

METHODS

Murine bone marrow-derived macrophages (BMDM) were polarized to a proinflammatory phenotype by IFN γ /LPS and infected with *Leishmania infantum* (*L. infantum*) MHOM/TN/80/IPT1 promastigotes (MOI 1:10). Nitric Oxide Synthase 2 (NOS2), Arginase 1 (ARG1) and Arginase 2 (ARG2) gene expression was evaluated by Real Time PCR after 6 hours in normoxia (20% O₂) or hypoxia (1% O₂); NO and urea levels (a measure of the activity of NOS or arginases) were evaluated in cell supernatants by Griess assay and by colorimetric urea assay after 24 hours in normoxia or hypoxia.

RESULTS

In normoxia, *L. infantum* infection reduced NOS2 expression and NO levels induced by IFN γ /LPS; concomitantly it increased ARG1 and urea levels, although a reduction of ARG2 expression. Despite hypoxia induced NOS2 expression, NO production was completely abolished in all conditions. ARG1 expression and urea levels were also increased by hypoxia. *L. infantum* infection reduced the expression of both NOS2 and ARG1 induced by hypoxia but with no significant effects on urea production.

CONCLUSIONS

In normoxia *L. infantum* infection promotes parasite survival increasing arginases activity to the detriment of NO production. Hypoxia also seems to favor the survival of the parasites modulating arginine metabolism in favor of arginases. A better comprehension of hypoxia and *Leishmania* infection interplay on the macrophage defense mechanism is crucial both for a better knowledge of leishmaniasis pathogenesis and in the perspective of new possible treatments.

P098

TRPM8: A NEW EMERGING TARGET FOR MELANOMA TREATMENT

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BACKGROUND-AIM

The incidence of melanoma is increasing worldwide. Despite the considerable efforts to find new therapies, sometimes patients acquire resistance and the disease progresses. Thus, the need of finding new intracellular targets emerges. The transient receptor potential melastatin subtype 8 (TRPM8) is a nonselective cation channel, with a certain preference for calcium permeation. This channel is aberrantly expressed in different malignant solid tumors including prostate, pancreatic cancers as well as melanoma. Although its role in melanoma is still unclear, this finding makes TRPM8 a potential valuable therapeutic target in melanoma cells.

METHODS

We have used two different melanoma cells derived by subcutaneous and lymph node metastasis and different approaches (colorimetric assays, western blot analysis, immunofluorescence (IF) microscopy) to analyze the biological effects of several TRPM8 modulators in melanoma cells.

RESULTS

Time course experiments (from 24 to 72 hours) have shown that high concentrations (1 μ M-10 μ M) of TRPM8 modulators induced cell death of two melanoma cell lines, the AMM16 and WM2664. IF approaches, by using a dual-fluorescence system, confirmed these data. Thus, we analyzed the molecular pathway involved in the apoptotic program induced by these drugs and we have shown that it is mediated by caspase-3 activation and proteolytic cleavage of PARP-1.

CONCLUSIONS

Further experiments are needed to clarify the molecular mechanism at the basis of the channel regulation and the consequent Calcium influx rate. However, the identification of new target chemotherapeutic options with improved potency and selectivity could be a novel strategy to treat melanoma. These novel therapeutical approaches are expected to have a great impact on the oncology field.

P099

THE COOPERATION BETWEEN THE ANDROGEN RECEPTOR AND FILAMIN A PREVENTS CELL SENESCENCE IN SKELETAL MUSCLE.

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BACKGROUND-AIM

Ageing induces a slow and progressive decrease in muscle mass and muscle functions, causing sarcopenia. Despite it is a common feature of geriatric syndrome, patients affected by sarcopenia are often neglected or poorly treated. Androgens play crucial physiological roles in male reproductive organs, but also in several extragenital structures including muscle and bone. Particularly, testosterone exerts important anabolic functions in skeletal muscle by engaging the androgen receptor (AR), activating both genomic and non-genomic pathways. Thus, the analysis of AR and its co-interactors could offer new hints to treat this disease.

METHODS

Skeletal muscle biopsies were analyzed by western blot (WB) and Co-Immunoprecipitation to investigate the AR expression and its complexation with Filamin A. Skeletal muscle cells, C2C12, were analyzed by WB, immunofluorescence (IF) and RGB microscopy approaches to analyze the AR expression, its localization and cellular senescence.

RESULTS

The analysis of Skeletal muscle biopsies derived by 20 subjects divided into two groups, young (<58 years; mean age 46,5) and elderly patients (>58 years; mean age 72,6; Table I) revealed that young subjects (ys) expressed higher levels of AR if compared to older subjects (os). In the same samples p16, a key regulator of the senescence program resulted to be more expressed in os than in ys (Figure 1). Furthermore, only in ys AR co-immunoprecipitates with FlnA (Figure 2). C2C12 cells express AR, that under basal conditions is prevalently localized in cytoplasm of cells and that upon 60 minutes of androgen stimulation enters into the nuclei of the cells (Figure 2). The androgen binding to AR is able to revert cellular senescence induced by H₂O₂. Noteworthy, the use of a stapled peptide displacing the AR/FlnA complex, RH-2025u, increases the cell senescence.

CONCLUSIONS

The disruption of the androgen/AR axis occurring in the skeletal muscle of aging subjects results in excessive metabolic functions and muscle loss.

Taken together our data show that FlnA is an important scaffold for the AR. We might suppose that the complex exerts a role in the maintenance of skeletal muscle homeostatic trophism avoiding cell senescence.

Further exploration in cultured cells is needed to promote new clinical interventions that may restore muscle function and enhance the clinical outcomes associated with age-related frailty and sarcopenia.

THE DIALOGUE BETWEEN PROSTATE CANCER AND CARCINOMA-ASSOCIATED FIBROBLASTS: THE INFLUENCE OF ANDROGENS AND NERVE GROWTH FACTOR

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BACKGROUND-AIM

Prostate cancer (PC) is the most diagnosed and the second most lethal cancer in men. At early stage, different treatments are available. When PC acquires a castration-resistant phenotype, usually culminating in the onset of metastases, few approaches are actionable, and the death toll remains high. PC development and progression occur with alterations in the surrounding tumor microenvironment (TME), made up of mesenchymal-derived cells (including carcinoma-associated fibroblasts, CAFs), resident or infiltrating vascular structures and an immune cellular component. PC cells can sculpt TME through the secretion of various factors, including androgens and nerve growth factor (NGF) that released by both epithelial PC cells and CAFs trigger the activation of various signalling cascades promoting the metastatic phenotype.

METHODS

CAFs, prepared ex vivo from a cohort of patients affected by PC at different Gleason's score, and different PC cells were used in 3D co-cultures. By biochemical and confocal microscopy approaches the relationships between the two compartments have been analyzed. Specific inhibitors and siRNA molecules have helped to clarify the signalling pathways involved in androgens- or NGF-induced biological effects.

RESULTS

PC cells release both androgens and NGF, thus recruiting CAFs through the binding to their cognate receptors expressed by CAFs. In turn, CAFs move towards PC cells thereby increasing PC organoid size. The crosstalk between the two compartments might be related to a paracrine loop. The androgens' induced effects are in part due to the establishment of AR/FlnA complex in both cellular compartments. The NGF-induced effects can occur through a cross-talk between AR/TRKα or TRKα/integrin β1/Src/FAK in the epithelial compartment. The signalling pathways elicited by NGF and involved in CAFs invasiveness are still to be elucidated.

CONCLUSIONS

Evidence correlates the NGF and androgenic axis in the brain, but also in some cancer types, including prostate and colon. An AR/TrkA crosstalk occurs in PC. A similar plot might be involved in its surrounding mesenchymal cells. Future investigations are needed to clarify the intracrinology in TME. Emerging data concerning the spreading of PC cells along nerves through the perineural invasion opens a new scenario since it seems responsible for most PC extra-capsular spreading. More in-depth studies might identify new therapeutic strategies to be used in combo with chemo- or immune-therapies to slow or stop PC progression.

EFFECT OF THE NOVEL THR β AGONIST TG68 ON CARDIAC FUNCTION IN A RAT NAFLD-ASSOCIATED HEPATOCARCINOGENESIS

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BACKGROUND-AIM

The burden of hepatocellular carcinoma (HCC), a tumor characterized by dismal prognosis and poor survival rate, has been increasing in patients with evidence of non-alcoholic fatty liver disease (NAFLD), the most common cause of liver disease in Western countries. The recent increased incidence of NAFLD and HCC has been associated with the alteration of the thyroid hormone/thyroid hormone receptors (TH/THRs) axis. By activating the THR pathway in hepatocytes, THs ameliorates hepatic steatosis and, in murine models of liver cancer administration of triiodothyronine (T3) exerts an anti-tumorigenic effect on both early and advanced HCC by inducing differentiation of neoplastic hepatocytes. Unfortunately, the use of T3 as therapeutic agent has been hampered by undesirable systemic side effects, the most harmful of which are cardiac side effects, such as heart failure and tachy-arrhythmias. These adverse effects are probably due to the activation of the α -isoform of THR, which is highly expressed in cardiomyocytes but not in hepatocytes.

For this reason the design of specific THR β agonists, possessing the hepatic beneficial effects of T3 in the absence of its toxic extrahepatic effects, has raised a great interest. On these premises, we recently investigated the effects of a novel THR β agonist, namely TG68, on preneoplastic liver lesions induced in a murine model of NAFLD-associated liver carcinogenesis. The results showed that TG68 causes a massive reduction of steatosis and a rapid regression of preneoplastic hepatic lesions but without causing overt heart dysfunction.

METHODS

The aim of this study was to investigate, by using an in vivo rat model and in vitro 2D and 3D cell cultures, whether the hepatic beneficial effects of TG68 were accompanied by molecular changes in cardiac functions, not excluding positive actions in contractile function.

RESULTS

Oral TG68 administration did not cause an increase in heart weight/body weight ratio, hypertrophy, and changes in genes and miRNAs involved in cardiac dysfunction in HFD-fed rats as compared to rats treated with T3. Preliminary in vitro results obtained with embryonic rat cardiomyocytes, namely H9C2 cells, contributed to further elucidate the mechanisms underlining the effect of thyromimetics on cardiac function.

CONCLUSIONS

The absence of deleterious cardiac effects following the treatment with TG68 strongly suggests the potential use of THR β agonists as a novel therapeutic strategy in NAFLD and NAFLD-induced HCC.

ANALYSIS OF HBV INTEGRATION IN MITOCHONDRIAL DNA OF HEPAD38 CELLS BY THE HIGH-THROUGHPUT HBV INTEGRATION SEQUENCING (HBIS) AND RNASEQ APPROACHES.

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BACKGROUND-AIM

Hepatitis B virus (HBV) infection is a leading cause of hepatocellular carcinoma (HCC) worldwide. The integration of HBV DNA into the host genome is one of the carcinogenic mechanisms of HBV. It has been recently demonstrated that mitochondrial DNA (mtDNA) can be a target of HBV, suggesting a new potential mechanism by which HBV integration may contribute to liver damage and HCC development. Aim: (1) To conduct a deeper investigation of HBV integration in mtDNA of HepAD38 cell line, which derives from HepG2 cells and supports tetracycline (Tet)-off inducible HBV replication; (2) to evaluate mitochondrial function in HBV-replicating HepAD38 cells.

METHODS

We used a high-throughput HBV integration sequencing (HBIS) approach and RNASeq to investigate HBV integration in mitochondria isolate from HepAD38 cells after Tet removal for 7 days. Moreover, at the same time point we analysed mitochondrial function of HepAD38 cells using the Seahorse XFp analyzer.

RESULTS

After 7 days Tet removal, mean amounts of HBV DNA and HBV RNA in HepAD38 cells were $1.1 \times 10^3 \pm 6.0 \times 10^2$ and $1.0 \times 10 \pm 5.9 \times 10^{-1}$ copies/cell, respectively; while mean amounts of HBV DNA and HBsAg in the cell supernatants were $2 \times 10^6 \pm 2.7 \times 10^4$ copies/mL and $4.1 \times 10^3 \pm 7.2 \times 10^2$ IU/mL, respectively. At the same time point, HBIS led us to detect a mean amount of 81 ± 11.9 HBV integration sites in mtDNA from HBV-replicating HepAD38. In particular, HBV integration sites were detected at high frequency in COX1, RNR2, and ND2 mitochondrial genes. In addition, RNASeq analysis led us to detect large amount (mean \pm S.D.: 635 ± 143.7) of HBV-mitochondria chimeric transcripts in HBV-induced HepAD38 cells. These transcripts contained - at higher frequency - sequences corresponding COX1, RNR2, ND2, ND4, and ND6 mitochondrial genes. The analysis of mitochondrial function showed that, compared to HepAD38 cells without HBV replication and viral integration, HBV replicating HepAD38 cells had a 2-fold reduction of basal respiration, of ATP-linked respiration, and of maximal respiration.

CONCLUSIONS

(1) HBV may integrate into mtDNA of HBV replicating HepAD38 cells; (2) The site of HBV insertion into mtDNA may be transcriptionally active; (3) HBV replication and viral integration into mtDNA may induce mitochondrial dysfunction.

DISSECTION OF HUMORAL IMMUNE RESPONSE AGAINST SARS-COV-2 NUCLEOPROTEIN IN COVID-19 PATIENTS

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BACKGROUND-AIM

In hospitalized COVID-19 patients an immune response towards SARS-CoV-2 nucleoprotein is described, but very few data are available on the epitope specificity, the avidity of anti-N antibodies and their value in predicting disease severity and outcome of infection.

The aim of this work was to elucidate/dissect anti-N immune response in COVID-19 patients

METHODS

Eighty two consecutive highly symptomatic COVID-19 patients were recruited at the Intensive Care Unit of the AOU Pisana. Anti-S and anti-N antibodies were evaluated by indirect ELISA on recombinant proteins. Epitope mapping of N protein was performed using seven 20-mer peptides chosen based on bio informatic analysis; anti-N peptides antibodies were evaluated by indirect ELISA. Anti N- and N-peptides antibody avidity was evaluated with a modified ELISA involving usage of chaotropic agents, such as Urea, NaSCN and NaCl.

RESULTS

Anti N IgG, IgA, and IgM were detected in 59%, 48% and 34% respectively.

Anti-N IgG is the most prevalent isotype and often coexist with anti-N IgA.

Anti-N IgG coexist with anti-S IgG in 51% patients. However, in 21% anti-S are present alone and in 10% of cases anti N are the only antibody present in COVID-19 patients.

Multivariate analysis displayed no correlations of anti-N antibodies with clinical progression or final outcome.

In anti-N positive patients, we analysed anti N derived peptides and found that N aa366-388 is the more frequently recognized sequence being bound by 33% Covid-19 patients.

Anti N immune response in COVID-19 patients is homogeneously characterised by high avidity: in fact Urea and NaCl do not have effects on anti-N antibodies (avidity index – AI=7.8 and AI>4, respectively), whereas antibody binding is inhibited by NaSCN (AI=2.1). On the contrary, anti-N aa366-388 avidity is more heterogenous, with patients displaying antibodies inhibited by NaCl (AI=1).

CONCLUSIONS

Anti nucleoprotein antibodies are present in COVID-19 patients, even in absence of anti-S antibodies and might be useful in evaluation of patient's immune response , where N aa366-388 peptide represents the immunodominant epitope. Antibody avidity may allow a finer typing of individual immune response. These data may be valuable for the development of subunit vaccines for COVID-19.

THE OSM/OSMR β SIGNALLING AXIS IN THE DEVELOPMENT OF MASLD-RELATED HEPATOCELLULAR CARCINOMA

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BACKGROUND-AIM

Oncostatin M (OSM) is a pleiotropic cytokine belonging to the IL-6 family that has been proposed to contribute to the progression of chronic liver diseases and hepatocellular carcinoma (HCC). Patients with HCC arising on a metabolic dysfunction-associated steatotic liver disease (MASLD) background showed increased OSM serum levels that correlate with clinical parameters and disease outcome. Accordingly, data from a murine model of MASLD-related HCC showed that OSM expression increased during liver carcinogenesis and correlated with F4/80 gene expression, suggesting an interplay between OSM and macrophage recruitment/functions in the tumour microenvironment. In this study, we investigated the potential contribution of OSM in the modulation of the tumour microenvironment by affecting crucial events of HCC progression.

METHODS

To explore such a hypothesis, we have employed: a) mice genetically modified to abrogate OSMR β expression into hepatocytes (OSMR β -/- mice) and wild-type (WT) littermates, that underwent the experimental DEN/CDAA protocol of MASLD-related liver carcinogenesis to investigate whether the disruption of the OSM/OSMR β signalling within hepatocytes may affect the development and progression of MASLD-related HCC; b) cohort of MASLD patients with/without HCC.

RESULTS

Data obtained indicate that the induction and progression of MASLD-related liver tumours in OSMR β -/- mice differ significantly from what was observed in WT mice as follows: i) a significant decrease in tumour masses, ii) a significant reduction of angiogenic switch, iii) a reduction in specific proliferation markers; iv) an impairment of the immunosuppressive landscape. Moreover, Multiplex Immunoassay performed on sera from MASLD cirrhotic and/or HCC patients revealed a significant increase of a subset of cytokines characterizing the immunosuppressive tumour microenvironment (IL1 β , CCL2, IL8, CXCL13). The transcript levels of these cytokines correlated with OSM expression in MASLD patients. Accordingly, the OSMR β -/- resulted in a reduction of the transcript levels of these specific genes.

CONCLUSIONS

Experimental data highlight a pro-carcinogenic contribution for OSM in MASLD by promoting a pro-tumorigenic microenvironment, suggesting a possible role for the OSM-OSMR β axis as a therapeutic target for MASLD-related HCC.

ANTI-CANCER EFFECT OF GLABRESCIONE B IN PRECLINICAL MODELS OF HEDGEHOG-DEPENDENT TUMORS: IMPLICATION FOR ITS TRANSLATION INTO CLINICAL PRACTICE

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BACKGROUND-AIM

Hedgehog (HH) signaling is one of the most important developmental pathways, whose aberrant activation is responsible for a wide spectrum of cancers, including medulloblastoma (MB) and basal cell carcinoma (BCC). Given its crucial role in tumorigenesis, the HH pathway has emerged as a promising target for the exploration of more efficient anti-cancer therapies. We identified Glabrescione B (GlaB), a natural compound found in *Derris glabrescens* (Leguminosae) seeds, as the first small molecule able to directly inhibit Gli1, the most powerful effector of HH signaling, showing excellent anti-cancer properties in HH-driven tumors. To overcome the poor water solubility and to enhance GlaB therapeutic efficacy, we recently explored several dissolution strategies, such as liposomes.

METHODS

Liposomes used for the encapsulation of GlaB were prepared by thin film hydration method. The effect of the liposomal composition was investigated in terms of loading efficiency/capacity of GlaB and liposomes dimension. The ability of GlaB formulated in liposomes (Lipo/GlaB) to inhibit the Gli1 transcriptional activity was determined by luciferase assay. The anti-cancer effect of Lipo/GlaB to impair HH-dependent tumor growth was examined in primary HH-MB cells and in a HH-dependent mouse model of BCC. Gli1 expression levels in tumor masses have been analyzed both by qRT-PCR and IHC staining.

RESULTS

GlaB formulated in liposomes shows a great ability to inhibit the Gli1 transcriptional activity in MEFs transiently expressing ectopic Gli1 and a Gli-dependent luciferase reporter. Moreover, Lipo/GlaB robustly suppresses in a dose-dependent manner the proliferation of primary tumor cells freshly isolated from Math1-Cre/Ptc^{-/-} mice, that spontaneously develop HH-MB. Importantly, Lipo/GlaB also shows a strong ability to inhibit the tumor growth in an in vivo model of HH-dependent BCC, which is associated with a consistent reduction of Gli1 expression levels.

CONCLUSIONS

Our findings reveal the excellent anti-tumor properties of GlaB encapsulated in colloidal carriers, namely liposomes, for the treatment of HH-driven tumors and provides relevant implications for the translation of GlaB into clinical practice.

RNA MEDICINE: A NOVEL APPROACH TO BLOCK THE PATHOLOGICAL COMMUNICATION BETWEEN TUMOR AND MICROENVIRONMENT.

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BACKGROUND-AIM

Multiple myeloma (MM) is a plasma cell malignancy in which monoclonal plasma cells proliferate in bone marrow (BM). The role of Notch aberrant activation in tumor development and progression has been established by many studies. The interplay between MM cells and the BM niche does not only rely on direct cell-cell interaction but a critical role is played by MM-derived extracellular vesicles (MM-EV). In fact, recently we proved that MM-EVs mediate the communication between MM and BM microenvironment in a NOTCH-dependent way. Due to the relevant role of Notch signaling in MM progression, we aim to provide the proof of concept for a siRNA-mediated therapeutic approach to inhibit Notch signaling in MM using EVs as a drug delivery tool. Here, we present a part of the methodological set-up.

METHODS

For the EV-mediated delivery set-up, EVs were isolated from the MM cell line by ultracentrifugation (110.000×g 75'). MM-EVs specific delivery to the tumor was assessed taking advantage of a xenograft mice model. For siRNA loading into EVs, we are taking advantage of sonication. The uploading setup is divided into 3 different steps 1) identification of best conditions of EV sonication using nanoparticle tracking analysis and FACs; 2) EVs sonication with siRNA, and 3) Evaluation of siRNA-loaded EVs up take in MM.

RESULTS

In vivo analysis of the xenograft mice was indicative of the tropism of the MM-EVs selectively recognizing the neoplastic tissue. Results of set-up indicate that sonication of the EVs does not significantly change their dimensional profile and quantity, while the uptake experiment showed that sonicated EVs can be uptaken less (~30%) in MM cells compared to the not sonicated ones.

CONCLUSIONS

In conclusion, the experiment of the xenograft model suggests that EVs can be used as a specific siRNA delivery tool. In addition, the preliminary results of the EVs sonication revealed that sonication does not significantly affect the EVs quality and quantity, but it can reduce EVs uptake by the MM-cells. In the future, we will sonicate EVs in the presence of siRNA and evaluate its efficacy and biological impacts in MM in vitro and in vivo.

KILLING CANCER CELLS WITH NATURAL COMPOUNDS: THE ACTIVITY OF GENISTEIN IN A GASTRIC ADENOCARCINOMA MODEL

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BACKGROUND-AIM

Gastric Cancer (GC) is one of the most widespread malignancies in the world being the 4th for incidence and the 5th cause of cancer death.

The absence of specific symptoms determines late diagnosis already at advanced stages. In these cases, surgery is the only decisive approach, supported by chemotherapy.

Nowadays, GC therapy involves the administration of fluoropyrimidines, taxanes and platinum compounds but frequently chemotherapy fails due to patient's resistance. So, the research of alternative methods to contrast tumor progression has led to study natural compounds that could support traditional therapy.

Genistein, a soy polyphenol, has been previously investigated for its capacity to slow cancer progression and dissemination and to induce cell death in liver, lung and colorectal cancer.

In this study we investigated the effect of Genistein on an in vitro model of Gastric Adenocarcinoma.

METHODS

We used the ACC 201 cell line and its resistant subpopulations, obtained by chronic single administration of 5-fluorouracil (5FU) or cisplatin (CISr) or paclitaxel (TAXr) and FLOT (FLOT) a combination of the previously named drugs. The effect of Genistein was evaluated by MTT assays and flow cytometry.

RESULTS

We first evaluated Genistein toxicity establishing a dose-response curve; in each tumor line we observed a greater reduction of viability compared to normal cells (fibroblast and healthy gastric mucosa).

The Annexin V-PI double staining analyzed in flow cytometry showed an increase of cell death in all lines. CISr had the highest value of apoptotic cells (23%) after treatment while the TAXr showed an increase in necrosis (from 5,86% to 26,9%).

This increase in cell death could be led by ROS production observed through the labelling with dichlorofluorescein diacetate (DCFDA).

The pro-apoptotic action of Genistein was also demonstrated by the increase of P53 after treatment in every cell line, where its expression is over 50%, except for WT and 5FU cells.

Moreover, in every chemoresistant cell line, we observed an increase of death after combined administration of Genistein and a sub-LD50 of each drug treatment.

CONCLUSIONS

These preliminary data show the efficacy of Genistein to promote apoptosis in adenocarcinoma cell lines; a further investigation could suggest a possible role as a coadjuvant in chemoresistant GC treatment.

SHORT-TERM TERT INHIBITION IMPAIRS CELL PROLIFERATION THROUGH THE NF- κ B/MYC/P21 AXIS IN IN VITRO AND IN VIVO MODELS: EVIDENCE FOR THE NON-CANONICAL FUNCTIONS OF TERT

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BACKGROUND-AIM

Besides its canonical role in stabilizing telomeres, TERT, the catalytic component of telomerase, may promote tumor growth/progression through extra-telomeric functions. Our previous in vitro and in vivo studies demonstrated that short-term telomerase inhibition by BIBR1532 (BIBR) impairs cell proliferation, with an accumulation of cells in the S-phase, without affecting telomere length. Here, we investigated the molecular mechanism(s) through which TERT inhibition impairs cell cycle progression in Epstein-Barr virus (EBV)-immortalized B-lymphoblastoid cell lines (LCLs), Burkitt's lymphoma (BL) cell lines, and in the zebrafish model.

METHODS

LCL and BL cells were short-term (24 hours) treated with BIBR. Expression of TERT, NF- κ B p65, MYC, and CDKN1A (P21) was evaluated in vitro at both the transcript and protein levels. The effects of Tert inhibition on Myc and p21 were analyzed in vivo in wild-type (WT) and tert mutant zebrafish embryos. Transfection experiments with vectors coding for WT TERT and biologically inactive TERT-HA in telomerase-negative U2OS cells were also performed.

RESULTS

Short-term TERT inhibition by BIBR in LCL and BL cells significantly reduced NF- κ B p65 nuclear levels, leading to the downregulation of a subset of NF- κ B target genes, including MYC. Interestingly, the NF- κ B inhibitor pyrrolidine dithiocarbamate also downregulated MYC expression and arrested the cell cycle in the S-phase. MYC downregulation induced by TERT inhibition was associated with increased expression and nuclear localization of P21, favouring its cell cycle inhibitory functions. Treatment of WT zebrafish embryos with BIBR significantly decreased Myc and increased p21 expression, while no effects were observed in tert mutant embryos. Notably, ectopic overexpression of both WT TERT and biologically inactive TERT-HA in telomerase-negative U2OS cells significantly increased the expression of NF- κ B target genes, including MYC.

CONCLUSIONS

These results provide evidence that TERT has telomere length-independent functions through NF- κ B p65 signaling and that telomerase inhibition can directly reduce the expression of NF- κ B target genes, including MYC, thus impairing cell proliferation both in vitro and in vivo.

HUR PROTECTS PD-L1 MRNA FROM MICRORNA-MEDIATED DEGRADATION UNDER HYPOXIAC. Costa ², M. Tathode ⁵, M. Caraglia ⁵, M. Cioce ⁴, L. Mutti ⁶, A. Giordano ³, F. Pentimalli ¹¹LUM University, Casamassima, Bari²National Cancer Institute of Naples, Pascale Foundation³Temple University, Philadelphia, USA⁴University Campus Bio-Medico Rome⁵University of Campania⁶University of L'Aquila**BACKGROUND-AIM**

Pleural mesothelioma is a deadly cancer caused by asbestos exposure. All mesothelioma histotypes have poor prognosis despite multimodal therapy. Recently, immunotherapy has been proposed as first line treatment. Various immunotherapy approaches are directed against PD-L1. We previously showed that in mesothelioma PD-L1 is targeted by the p53-regulated mir-320a, which is downregulated in tumors, along with mir-145. Here, we aimed to assess whether also mir-145 acted within the same p53-microRNAs-PD-L1 axis and to explore possible strategies to downregulate PD-L1 expression.

METHODS

As a model of mesothelioma, we used 2 cell lines derived from biphasic (MSTO-211H) or epithelial (NCI-H28) histotype and confirmed the results on a series of 64 archival specimens. MiRNA modulation was performed through commercial mimics; p53 was overexpressed by transient transfection and HUR was silenced by a plasmid expressing short hairpin RNAs.

RESULTS

Overexpressing mir-145 in MSTO-211H, reduced the levels of PD-L1 mRNA and protein. To assess whether mir-145 is controlled by p53 in mesothelioma, we transfected MSTO-211H and NCI-H28 with a p53-HA expressing construct and found that mir-145 was upregulated. In silico analysis confirmed that p53 and PD-L1 are anti-correlated and high PD-L1 expression associates with worse patient survival. We then analysed p53, PD-L1 and mir-145 expression by real time RT-qPCR in a series of 64 mesotheliomas vs normal mesothelium. We found that PD-L1 and TP53 were significantly anticorrelated ($r=-0.213$; $p<0.05$) as were miR-145 and PD-L1 ($r=-0.066$).

As mesothelioma is highly hypoxic, we treated cells with CoCl₂ to mimic hypoxia. CoCl₂ triggered the expression of HIF1 α and also of PD-L1 and p53. Interestingly, p53 activation effectively induced mir-145 and the other microRNAs previously shown to target PD-L1: mir-34, mir-200 and mir-320a. Nonetheless PD-L1 was expressed at high levels suggesting that it was protected by miRNA-mediated degradation. We then searched in silico factors that could protect PD-L1 and found that the RNA binding protein HUR could be a good candidate because it was reported to interact with all members of this miRNA-PD-L1 network. HUR silencing was able to reduce PD-L1 expression in both mesothelioma cell lines under normoxia and hypoxia.

CONCLUSIONS

Our data identify HUR as a therapeutic target to reduce PD-L1 in mesothelioma and counteract not only its inhibition of the antitumoral immune response but also its cell autonomous tumorigenic properties.

MITOCHONDRIAL DYSFUNCTION IN ECTOPIC CALCIFICATION.

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BACKGROUND-AIM

Ectopic calcification (EC) is due to an inappropriate mineralization occurring in soft connective tissues. EC is associated with i) elevated calcium and/or phosphate serum levels (e.g., chronic kidney diseases), ii) necrotic areas (e.g., atherosclerosis) or iii) altered balance of pro-osteogenic/anti-calcifying factors (e.g., genetic diseases). Pseudoxanthoma elasticum (PXE), for instance, is an autosomal recessive disease characterized by elastic fibers' calcification in various tissues/organs (e.g., in skin, eyes and blood vessels). Interestingly, a mild chronic oxidative stress condition was demonstrated in PXE patients as well as in vitro cultured fibroblasts. Oxidative stress is generally due to an imbalance between a reduction of cellular antioxidant defense mechanisms and/or to an increased production of reactive oxygen species mainly generated in the mitochondria via electron transport chain. Since oxidative stress has been demonstrated to be a key player in vascular calcification, we have investigated structure and function of mitochondria from PXE dermal fibroblasts.

METHODS

LC-MS/MS was applied to investigate the proteomic profile of mitochondria from cultured fibroblasts isolated from skin biopsies of PXE patients and of healthy subjects. Oxygen consumption rate was measured using Seahorse XFe24 Analyzer and mitochondrial function was assessed using Mito stress test kit. By confocal microscopy cellular mitochondrial network and mitochondrial membrane potential were evaluated using MitoView Green and JC-1, respectively.

RESULTS

Mitochondrial proteome reveals several differentially expressed proteins involved in redox balance and in oxidative phosphorylation, PXE mitochondria demonstrate a low ability to cope with a sudden increased need for ATP and exhibit a more abundant, branched, and interconnected mitochondrial network compared to control cells.

CONCLUSIONS

Data indicate that PXE is characterized by dysfunctional mitochondria which have been associated to inhibition of the anti-calcifying factor ectonucleotide pyrophosphate/phosphodiesterase 1, thus favoring the mineralization of soft connective tissues. Thus, maintaining mitochondrial homeostasis (i.e., function and metabolism) could help protecting cells against calcification process.

SITE-SPECIFIC ANTAGOMLR DELIVERY AS AN INNOVATIVE THERAPEUTIC APPROACH FOR THROMBOINFLAMMATION ASSOCIATED WITH BEHÇET'S SYNDROME - MIRLIVERY

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BACKGROUND-AIM

Behçet's syndrome (BS) is a rare systemic vasculitis associated with vascular manifestations of inflammatory nature that make it a unique model of thrombo-inflammation unresponsive to traditional anticoagulation therapy.

Several studies suggested an active role of microRNAs (miRNAs) in complex immune-mediated disorders such as vasculitides.

Developing oligonucleotide sequences capable of selectively binding deregulated miRNAs (antagomiRs) at endothelial level, influencing their activity as gene expression modulators, may therefore represent a new frontier in the targeted therapy of BS-associated vascular complications.

The MIRLIVERY project aims to develop and test the efficacy of an innovative therapeutic strategy based on antagomiRs, embedded in immunoliposomes coated with nanobodies directed against VCAM1 (a molecule selectively expressed at the level of the endothelium activated in a pro-inflammatory sense). These functionalized immunoliposomes will act as carriers for specific antagomiRs, allowing them to be delivered to the endothelial level, providing site-specific targeted inhibition of target miRNAs.

METHODS

Differentially-expressed miRNAs in BS have been identified and validated using microarray technology and RT-qPCR. The therapeutic activity of selectively designed antagomiRs has been preliminary evaluated in an in vitro model of activated endothelium based on HUVEC cells stimulated with TNF- α .

RESULTS

Three selectively upregulated miRNAs in patients with BS have been identified. Based on in silico analysis, these miRNAs are involved in leukocyte perivascular infiltration, oxidative stress generation, and the modulation of the coagulation cascade. Our activated endothelium model has been developed and optimized. The three specific antagomiRs have been designed and preliminary tested on our in vitro model in terms of cell viability assessment and effect on endothelial activation markers, such as enhanced VCAM-1 exposure on the cellular membrane.

CONCLUSIONS

This study will contribute to the further definition of the potential of omics approaches as a source of information for the study of systemic vasculitides pathogenesis and for the design of innovative therapeutic strategies.

IDENTIFICATION OF AN INTERPLAY BETWEEN ERK5 AND HYPOXIA INDUCIBLE FACTOR 1 α IN INTRAHEPATIC CHOLANGIOCARCINOMA CELLS.

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BACKGROUND-AIM

Cholangiocarcinoma (CCA) is the second most common liver cancer after hepatocellular carcinoma, and constitutes a heterogeneous group of malignancies that arise from the epithelium of the biliary tree. In particular, intrahepatic cholangiocarcinoma (iCCA) is an aggressive liver malignancy with limited therapeutic options, and its incidence is increasing in the Western countries. Recently, our group reported the importance of the mitogen-activated protein kinase extracellular signal regulated kinase 5 (ERK5) in supporting the survival and proliferation of CCA cells both in vitro and in vivo. In order to identify additional molecular targets in CCA, we investigated on the possible functional relationship between ERK5 and hypoxia inducible factors α , the main regulators of the response to hypoxia, a condition that is typical of the tumour microenvironment.

METHODS

Two CCA cell lines (CCLP-1 and HUCCT-1) were grown at different time points under normoxia and hypoxia conditions. Gene silencing was performed with short harpin RNA for ERK5 gene (MAPK7). Protein expression analysis was investigated by Western Blot. For the pharmacological treatments, HIF and ERK5 inhibitors effects were evaluated in term of cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

RESULTS

We found that ERK5 phosphorylation and HIF1 α expression are increased in hypoxia. The increased activity of the latter in hypoxia was confirmed by the consistent increase of its target genes, Carbonic Anhydrase 9 (CAIX) and Glucose transporter 1 (GLUT1). We also found a functional relationship between ERK5 and HIF-1 α ; indeed, following ERK5 knockdown the increase of CAIX and GLUT1 was reduced in hypoxia compared to that observed in control cells (shNT). Combined treatment of ERK5i and HIFi in vitro and found a greater effect than the single treatments in hypoxic conditions. All together, these results lead to the idea to deepen this co-therapy to treat CCA, given the low oxygen concentration in tumor environment.

CONCLUSIONS

These findings led to the identification of a functional relationship between ERK5 and HIF-1 α in the regulation of CCA homeostasis, and put light on a new possible therapeutic option for CCA.

THE POTENTIAL ANTICANCER ACTIVITY OF DONKEY MILK IN HUMAN GASTRIC ADENOCARCINOMA (AGS) CELL LINE

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BACKGROUND-AIM

Human breast milk (HM) is considered an irreplaceable source of nutrition for newborn growth and its sensory and cognitive development. When breastfeeding is unavailable, the use of donkey milk (DM) can represent a good replacement due to its palatability, close chemical composition with HM and clinical tolerability mainly through its low caseins and minerals content. Currently, DM is known for its potential antimicrobial, antioxidant, antidiabetic, anti-inflammatory and immunomodulatory activities due to its bioactive compounds (i.e., fatty acids, whey proteins, lysozyme, immunoglobulins). Several studies have shown the beneficial effects of milk in the treatment of gastrointestinal diseases and their critical and significant role in the growth and maturity of gastrointestinal tract in infants. Therefore, our study aims to relate DM composition with the effects on a model of human gastric adenocarcinoma (AGS) cell line, in order to verify whether it affects cell viability.

METHODS

Fresh DM samples (pooled and at early, intermediate, late stage of lactation) were obtained from a local donkey farm (Ciucolandia, Capistrano, Italy). Milk samples from the three different stages of lactation were analyzed for total solids (TS), crude protein (CP), fat and lactose (LAC) content (IDF 141, 2013). Raw milk samples were skimmed by centrifugation. The effects of DM samples on AGS cell viability and migration were tested by MTS assay and wound healing assay, respectively.

RESULTS

According to literature data, the overall DM composition averaged 8.76(±0.14, SD) g/100g, 1.57(±0.05) g/100g, 0.38(±0.04) g/100g, 6.49(±0.06) g/100g for TS, CP, fat, and LAC, respectively. MTS assay results exhibited a dose-dependent anti-proliferative activity for DM samples on AGS cells as compared with control. DM samples from intermediate and late lactation exerted the higher cytotoxic effects at 100% concentration, after 72h. Furthermore, DM treatments were able to moderately reduce the capacity of cell migration compared to untreated AGS cells.

CONCLUSIONS

The preliminary results of our study suggest the potential anti-cancer effect of DM in a model of gastric cancer cell line and represent an interesting basis for further studies aimed at finding alternative therapies against cancers.

KCTD1 IS A NEW MODULATOR OF THE KCASH FAMILY OF HEDGEHOG SUPPRESSORS

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BACKGROUND-AIM

The KCASH oncosuppressor family are involved in the negative regulation of Sonic Hedgehog (Hh) pathway that plays a critical role in developmental processes and, when deregulated, may contribute to several cancers. KCASH1 and KCASH2 bind Cullin 3 to form a E3 ubiquitin ligase complex which can recruit and ubiquitinate HDAC1, inducing its proteasome-dependent degradation and thus blocking the activation of the main effector of the Hh pathway, Gli1. It was recently demonstrated that the KCTD15 is able to interact with KCASH2 protein, enhancing its negative effect on Hh pathway. KCTD1 shares several features and biological functions with its paralogue KCTD15, suggesting their common function on the modulation of Hh pathway.

METHODS

The potential KCTD1 role in Hh-dependent tumor is evaluated by in silico analysis from public databases. The ability of KCTD1 to decrease significantly the Gli1 activity was verified by Luciferase assays, RT-qPCR and WB analysis. Furthermore, through WB analysis and co-immunoprecipitation assays, we demonstrated that KCTD1 is able to bind and stabilize KCASH1 and KCASH2. Lastly, we evaluated that KCTD1 inhibits KCASH1 and KCASH2 proteasome-dependent degradation through the ubiquitination assay.

RESULTS

We demonstrated KCTD1 interaction with KCASH1 and KCASH2 proteins, and its role in their stabilization by reducing their ubiquitination and proteasome-mediated degradation. Consequently, KCTD1 expression reduces HDAC1 protein levels and Gli1 activity, inhibiting the cell proliferation in Hh tumor cells. Furthermore, analysis of expression data on publicly available databases indicates that KCTD1 expression is reduced in Hh-dependent Medulloblastoma samples, compared to normal cerebella, suggesting that KCTD1 may represent a new putative target for therapeutic approaches against Hh-dependent tumor.

CONCLUSIONS

The ability of KCTD1 to act as a modulator of both KCASH1 and KCASH2 could be a tool to broaden the current knowledge of the complex Hh signaling pathway. These information may constitute new bases for the identification of possible therapeutic approaches that increasing the expression and stability of KCASH proteins, downregulate the Hh pathway in Hh-dependent tumor types.

STUDY OF THE EFFECTIVENESS OF A NEW POLYCOMPONENT FORMULATION ON HYDROGEN PEROXIDE-INDUCED AGING IN HUMAN DERMAL FIBROBLASTS

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BACKGROUND-AIM

Human skin aging is a progressive process in each skin layer. Still, the most relevant cellular and molecular underlying mechanisms are associated with the aging of dermal fibroblasts and altered extracellular matrix homeostasis. Recently, the availability on the market of an innovative polycomponent formulation (K) containing a high molecular weight non-crosslinked-hyaluronic acid, a human recombinant polypeptide of collagen 1 alpha chain, and carboxymethyl cellulose, mainly used as single agents for skin rejuvenation, has addressed our scientific interest in evaluating its biomolecular effects on the in vitro fibroblast aging model.

METHODS

Normal human dermal fibroblasts (NHDFs) were first exposed to H₂O₂ to induce the aged phenotype, verified through the senescence-associated β -galactosidase staining. Aged fibroblasts were then cultured at different time intervals with increasing K concentrations. The proliferation rate was measured using the IncuCyte® system. Intra- and extracellular collagen I levels were assessed by western blotting, immunofluorescence, and Elisa. The expression of P4HA1, a protein involved in collagen synthesis, and α -SMA was also evaluated. The fibroblast contractility was assessed using the collagen gel retraction assay. TGF- β 1 levels were assayed by Elisa kit. The oxidative stress markers, including reactive oxygen species (ROS) and malondialdehyde (MDA), were also evaluated with standard methods.

RESULTS

The treatment with K counteracted, in a dose-dependent way, the H₂O₂-induced fibroblast aging, improving the proliferation rate and reducing β -galactosidase levels. An increase in collagen synthesis, α -SMA and TGF- β 1 levels, and contractility of aged HDFs was also registered after K treatment. Moreover, exposure to K formulation neutralized the aging-associated increase of MDA and ROS levels.

CONCLUSIONS

The obtained results suggest that the appropriate combination of an innovative formulation with three bioactive molecules with different individual mechanisms of action exerts on human dermal fibroblasts a synergistic effect in contrasting aging-associated biomolecular events.

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IDENTIFICATION OF SIDE POPULATION CELLS IN HUMAN LUNG CANCER (A549 CELL LINE) AND PRELIMINARY EVALUATION AFTER TREATMENT WITH STILBENES

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BACKGROUND-AIM

Lung cancer is one of the most clinically challenging tumour due to the aggressive proliferation, metastasis and the presence of cancer stem cells (CSCs). Many studies show the importance of consuming natural bioactive substances to prevent chronic diseases like cancer. Resveratrol (RSV) is a stilbene-based compound with wide biological properties including cancer chemoprevention activity, and its analogue 4,4'-dihydroxy-trans-stilbene (DHS) has shown superior activity in vitro and in vivo. Here, the effect of DHS and RSV was analysed on the A549 CSCs isolated in the side population (SP).

METHODS

A549 were FACS-sorted into main population (MP) and SP using Hoechst 33342, with Verapamil as negative control. The proliferation status (Ki67, MTT) and SOX2 expression by Western blot were analysed in SP, MP and non-sorted cells (NS). Clonogenic and soft agar assays were run for the 3 populations treated with 7.5-15-30 μ M RSV & DHS to assess anchorage dependent and independent growth.

RESULTS

The sorted SP (1.4%) kept in culture showed a different morphology and stronger SOX2 expression ($p < 0.05$) than MP & NS cells, confirming stemness. Proliferation evaluated by MTT & Ki67 did not show difference in growth. Treatment with DHS and to a lesser extent with RSV induced a dose-dependent reduction in clonogenic efficiency and colony formation in soft agar ($p < 0.05$), in particular in the SP population.

CONCLUSIONS

DHS affected CSCs more strongly than RSV, with further molecular characterization underway, alongside analyses in other cancer cell lines or tissues.

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ROLE OF PIN1-NOTCH3 AXIS IN SUSTAINING PLATINUM RESISTANCE IN OVARIAN CANCER

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BACKGROUND-AIM

Ovarian Cancer (OC) is one of the most lethal female-related diseases. Primary cytoreductive surgery and platinum (PT)-based chemotherapy represent the standard treatments for OC-bearing patients. Nevertheless, the development of drug resistance challenged the efficacy of the therapies, thereby highlighting that tackling PT-resistance is an urgent need in OC. Interestingly, Notch receptors are involved in PT-resistance. Notch3 (N3) is altered in a wide panel of OC, and it confers PT-resistance to OC cell lines. Therefore, effective targeting of N3 may restore Platinum sensitivity. We previously demonstrated that Pin1 positively regulates N3 in T-cell leukemia and we investigated this relationship also in OC context. Overall, in the present study we wondered whether and how Pin1-Notch3 axis is involved in PT-resistance in OC

METHODS

We used a wide panel of established OC cell lines. We performed: 1. in silico analysis on protein data collected by OC-bearing patients; 2. immunohistochemistry (IHC) on OC tissue samples; 3. long-term PT treatments for the generation of isogenic PT-resistant OC cell lines; 4. lentiviral transductions for the generation of N3-overexpressing cell lines; 5. in vitro studies such as pharmacological treatments, cell viability and colony formation assays; and 6. in vivo studies: xenograft experiments in NOD/SCID immuno-deficient mice

RESULTS

We first documented that N3 is involved in PT-resistance in vitro and in vivo by performing experiments on stable N3-overexpressing clones. We next demonstrated that PT-treatment induced an increased Pin1-Notch3 endogenous interaction, finally resulting in N3 stabilization. Consistently, Pin1 inhibition restored PT-sensitivity in vitro. Moreover, IHC on primary lesions from OC-bearing patients revealed that a consistent proportion of patients displays high expression of both proteins, which is also consistent with a poor prognosis

CONCLUSIONS

These observations strongly suggest that Pin1-Notch3 axis might represent a novel "tunable" molecular pathway in Ovarian Cancer to maximize PT efficacy.

ENHANCING ANTITUMOR EFFICACY OF AFATINIB WITH CURCUMIN IN MALIGNANT MESOTHELIOMA: A PROMISING COMBINATION STRATEGY

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BACKGROUND-AIM

Malignant mesothelioma (MM) is a rare and aggressive tumor that develops from the mesothelial cell lining of serous membranes. Several attempts have been made to develop targeted therapies aimed at improving the presently poor prognosis for this tumor. In this context, we evaluated whether the polyphenol Curcumin (CUR) may boost the anticancer efficacy of the ErbB receptors inhibitor Afatinib (AFA) on MM. The rationale behind this hypothesis was that CUR could prevent the activation of alternative "bypass" pathways able to lower MM cells sensitivity to the cytotoxic effects of AFA.

METHODS

The effects of AFA and CUR, alone or in combination, on cell proliferation, cell cycle regulation, apoptosis, autophagy and modulation of pro-survival signaling pathways were evaluated in cultured human and mouse MM cell lines with different histotypes. The in vivo anti-tumor activity of the compounds was investigated in mice bearing intraperitoneally transplanted, syngeneic MM cells.

RESULTS

As assessed by SRB assays and FACS analysis, CUR was able to potentiate the effect of AFA and the stronger antitumor effect of the drugs combination was associated either with an increased growth inhibition or with an increased apoptosis. The stronger pro-apoptotic effect of the drugs combination was observed in the cell lines with an epithelioid phenotype. CUR appeared to potentiate the effects of AFA by acting downstream of ErbB receptors. The combined treatment was more effective than AFA in decreasing phospho-ERK2 levels as well as the phosphorylated levels of the pro-survival kinase AKT only in human MM cells with epithelioid features. These results indicate that a strong potentiation of AFA pro-apoptotic effects by CUR may involve a synergistic inhibition of ERK2. In in vivo studies, the combined treatment significantly increased mice mean survival as compared to the treatment with the individual compounds.

CONCLUSIONS

Even though the mechanisms underlying the interaction between CUR and AFA appear to be histotype-dependent, the combination of AFA and CUR can have a stronger impact on MM progression as compared to the effect of the individual compounds, which can be ascribed either to increased growth inhibition or to an enhanced pro-apoptotic effect.

UNCOVERING THE MOLECULAR MECHANISM GOVERNING PIN1-WWP2 ANTAGONISTIC INTERPLAY ON NOTCH3 IN OVARIAN CANCER

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BACKGROUND-AIM

Ovarian Cancer (OC) curative therapy and survival trends have not significantly changed over the past decades, because of the lack of a definitive screening tool and vague symptoms or because of the insurgence of recurrence connected with resistance to treatments. Therefore, the identification and characterization of novel therapy targets are required. The Notch3 (N3) signaling pathway holds promise in being a candidate for novel specific targeted therapies given its role in tumor development and progression also in OC. Consequently, the exploitation of N3 post-translational modifications (PTMs) is rapidly emerging as an innovative approach, thus allowing the prediction of the interaction between N3 and its regulators. In this backdrop, the appealing candidates to fine-tune N3 stability are the isomerase Pin1, previously identified as a N3-positive regulator in T-cell acute lymphoblastic leukemia, and the E3 ligase WWP2, known to ubiquitinate N3 in OC context. However, what impact each interactor may have on N3 and on each other is still largely unknown. Our results supported the hypothesis that Pin1 is able to hinder the activity of N3-negative regulator WWP2.

METHODS

Therefore, we performed several in vitro studies in exogenous system to dissect in detail the molecular mechanism

RESULTS

We demonstrated 1. The WWP2 activity on N3 protein, focused on N3 intracellular domain (N3ICD), and 2. The Pin1-WWP2 potential antagonistic interplay on N3ICD.

CONCLUSIONS

Overall, these data will help us to deepen our understandings of N3 post-translational regulation, thus identifying new molecular targets on Pin1 and WWP2 competition, aimed at developing novel therapeutic strategies for OC-bearing patients.

INTRACELLULAR PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 AND INFLAMMATION IN ATHEROSCLEROSIS: IN VIVO AND IN VITRO EVIDENCE

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BACKGROUND-AIM

Atherosclerosis is the primary cause of cardiovascular disease, the leading contributor of morbidity and mortality worldwide. Each stage of the life cycle of atherosclerotic plaques is orchestrated by inflammation. PCSK9 could be placed at the basis of the pathogenic mechanisms of atherosclerosis, with potential effects independent of cholesterol levels. Although a variety of evidence demonstrates that PCSK9 plays a role in atherosclerotic inflammation, the direct mechanism of involvement is still unknown. Hypothesizing a role for extracellular vesicles (EVs) as possible mediator, aim was to unveil the impact of EVs derived from human smooth muscle cells (hSMCs) overexpressing PCSK9 on the inflammatory milieu.

METHODS

EVs isolated from hSMCs overexpressing or not PCSK9 (EVs^{PCSK9} and EVs^{CTR}); zebrafish; THP-1 and derived-macrophages; EA.hy926 endothelial cells; FACS; WB; nanoparticle tracking analysis; TEM; proteomic and miRNAs analysis; mitochondrial respiration.

RESULTS

hSMCs switched towards a synthetic phenotype when overexpressing PCSK9. EVs isolated from hSMCs^{PCSK9} were themselves enriched in PCSK9. Their systemic injection in zebrafish embryos led to a dose-dependent increased expression of proinflammatory cytokines (IL-1 β and IL-8), while their local injection to a recruitment of macrophages. The study of EVs cargo through a proteomic analysis identified 14 proteins significantly more abundant in EVs^{PCSK9}, highlighting an enrichment in extracellular matrix structural constituents. A miRNome analysis showed 6 differently expressed miRNAs targeting genes associated with atherosclerosis and inflammation. The evidence discovered in vivo was confirmed also in in vitro models. In monocytes and derived-macrophages, 24-h exposure to EVs^{PCSK9}: (i) raised the gene expression of MCP-1, IL-1 α and β , IL-6, and IL-8; (ii) raised the phosphorylation of STAT3 and decreased that of SOCS3; (iii) raised the uptake of oxLDL; (iv) decreased the mitochondrial respiration; (v) increased the migratory capacity. Inflammation was also raised when endothelial cells (EA.hy926) were treated with EVs^{PCSK9}.

CONCLUSIONS

EVs enriched in PCSK9 appear to favor a pro-atherogenic inflammatory phenotype.

CHECKPOINT MECHANISMS UNDERLYING ILC2 ACTIVITY

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BACKGROUND-AIM

Innate lymphoid cells (ILCs) provide protection at barrier interfaces and help maintaining the epithelial integrity. Innate lymphocytes are classified into five prototypical subsets, named NK cells, ILC1, ILC2, ILC3 and lymphoid tissue inducer (LTI) cells. ILC2 are tissue resident cells able to quickly produce type 2 cytokines, providing protection against parasites. ILC2 depend on the TFs Gata3 and RORa, which are indispensable for development and survival of ILC2. During helminth infection, ILC2 migrate to the lung, acquiring an inflammatory phenotype and the ability to produce IL-17. While the processes driving ILC2 differentiation and activation have been extensively dissected, our understanding of how ILC2 responses are turned off is still poorly understood. The project aims to dissect the checkpoint mechanisms triggered upon activation of ILC2.

METHODS

For the IL-25 experiment, C57BL/6J mice were injected with recombinant IL-25 +/- an anti-mouse CTLA4 daily for three days. For the in vitro experiments, ILC2 were sorted from the small intestine of C57BL/6J mice and cultured in presence of activating cytokines. Flow cytometry analysis was used to characterize ILC2 subset ex vivo or upon in vitro culture. The R package was used for the scRNAseq analysis, while DNA-accessibility of Ctla4 locus was visualized using IGV.

RESULTS

To identify checkpoint mechanisms of ILC functions, we analyzed available RNA-seq data of murine ILCs in distinct settings. We found that, Ctla4 transcript was one of the most induced inhibitory receptors in activated ILC2 isolated from the lung of *N. brasiliensis* infected mice. Induction of Ctla4 transcript occurred along with increased DNA accessibility of the Ctla4 locus. By employing both an in vitro system based on cytokine stimulation as well as an in vivo model based on administration of IL-25, we showed that activated ILC2 expressed CTLA4 also at protein level. To begin to understand the role of CTLA4 on ILC2 activation, we administered a blocking CTLA4 antibody to mice receiving IL-25, which induced ILC2 differentiation toward a proinflammatory/pathogenic phenotype characterized by loss of IL-33 receptor expression and higher IL-17 levels. These experiments suggested a direct role for CTLA4 expression in regulating ILC2 functions.

CONCLUSIONS

ILC2 express CTLA4 upon activation through a mechanism involving changes of DNA-accessibility and the action of multiple cytokines. Moreover, our data suggest a role for CTLA4 in regulating ILC2 activation.

THE NEW EMERGING ROLE OF NLRP4 INFLAMMASOME PLATFORM AS REGULATOR OF TBK1 AND AKT IN T47D BREAST CANCER CELL LINE.

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BACKGROUND-AIM

The crosstalk between inflammasome and cancer has been investigated extensively. NLRP4 forms inflammasomes and is a member of (NOD)-like receptors (NLRs) family. This protein acts as a negative regulator of TBK1, a serin/threonine protein kinase, by ubiquitination and proteasomal degradation. Moreover, TBK1 has a critical role in tumor development activating the kinase AKT signaling pathway. This evidence prompted us to investigate the potential protective role of NLRP4 during tumorigenesis, through the degradation of TBK1 as targetable link supporting context-selective mobilization of AKT regulatory network.

METHODS

Cell culture and treatments, transient and stable transfection, western blotting, qRT-PCR, focus formation assay, growth curves and statistical analysis.

RESULTS

Our results showed that protein expression of NLRP4 is higher in control cell line (MCF10A) compared to breast cell lines (MDA, T47D and MCF7). T47D revealed the lowest protein level of NLRP4. Instead, TBK1 protein showed high level in breast cell lines, especially in T47D. Level of NLRP4 mRNA in T47D ($p < 0,01$) confirmed the protein levels. Transient transfection of NLRP4 in T47D (T47D NLRP4 myc-DDK) lowered TBK1 protein levels ($p < 0,05$) when compared to T47D transfected with empty vector (T47D myc-DDK, control). Moreover, p-AKT (T308) decreased while the level of AKT protein did not modify in the same transfection assay. Stable T47D transfected cell with NLRP4 or control vector were treated with cycloheximide to evaluate TBK1 protein degradation in presence of NLRP4. The increased amount of NLRP4 protein reduced TBK1 stability. This result confirmed that NLRP4 promotes TBK1 degradation. For a greater understanding about the role of NLRP4 in tumor process, we analyzed the phenotypic effects of NLRP4 overexpression. A decreased cell growth was observed in T47D NLRP4 myc-DDK compared to T47D myc-DDK. Focus formation showed the potential tumor suppressor activity of NLRP4 ($p < 0,05$).

CONCLUSIONS

In brief, our results showed that NLRP4 has a role in the activation of TBK1, a crucial protein in the tumoral process. This link between these two proteins discloses a protective role of NLRP4 inflammasome during tumorigenesis.

A MESOPOROUS SILICA-BASED NANODEVICE AS A VEHICLE FOR THE HIGHLY SELECTIVE DELIVERY AND THE IMPROVED EFFICACY OF RESVERATROL AND DIHYDROTESTOSTERONE COMBINATION TO FOLATE RECEPTOR EXPRESSING BREAST CANCER CELLS

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BACKGROUND-AIM

Breast cancer (BC) is one of the principal causes of death among women. Besides surgery and radiation, hormone therapy, chemotherapy and targeted therapies represent the main therapeutic. However, side effects and drug resistance pose a long-term challenge to a patients' health. Therefore, the development of new therapeutic strategies specifically targeting cancer cells, sparing healthy cells is highly desirable. The syntheses and characterization of mesoporous silica nanoparticles (MSNs) grafted with folic acid (FOL) as a targeting function, as a multimodal nanovehicle for the targeted and pH-sensitive delivery of resveratrol (RSV) and dihydrotestosterone (DHT) to folate receptor positive (FR+) BC cells (FOL-MSN-RSV and FOL-MSN-DHT, respectively) were carried out.

METHODS

Efficacy studies were conducted by means of growth experiments. Seahorse analysis was performed to assess metabolic behavior in response to MSNs treatment and confirmed by Western Blotting (WB) analysis. WB was also used to evaluate the effect of the two prototypes and their combination on the expression of the main BC marker and therapeutic target, i.e. the estrogen receptor α (ER α).

RESULTS

The combination of FOL-MSN-RSV and FOL-MSN-DHT was able to selectively kill FR+ BC cells through ER α suppression and modulation of cell cycle regulatory proteins such as cyclin D1 (CD1), p21 and p27. Metabolic investigations showed that, compared to the treatment with the single prototype or the free drugs, FOL-MSN-RSV+FOL-MSN-DHT reduce the expression of mitochondrial TOM20, a central component of the TOM (translocase of outer membrane) receptor complex, as well as of several subunits belonging to the enzymatic complexes of the electron transport chain (OXPHOS). As observed with the combination of the two drugs, also FOL-MSN-RSV+FOL-MSN-DHT significantly affected the glycolytic metabolism and induced mitochondrial dysfunction. Notably, the vehicle alone (FOL-MSN) did not affect any biological process in both FR+ and FR- cells.

CONCLUSIONS

These data show the striking specificity of FOL-MSN-RSV+FOL-MSN-DHT toward FR+ BC cells and the outstanding safety of the FOL-MSN vehicle, paving the way for a future exploitation of these two combined prototypes in BC target therapy

ANDROGEN RECEPTOR-MEDIATED SIGNALING INFLUENCE BCL-2 AGONIST OF CELL DEATH (BAD) EXPRESSION AND FUNCTION IN BREAST CANCER CELLS.

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BACKGROUND-AIM

Androgen receptor (AR) expression in breast cancer (BC) growth and progression is clinically relevant and disease context specific. In estrogen receptor (ER) α -positive (ER α +) primary BCs, AR positivity correlates with lower tumor grade and a better clinical outcome. These clinical-pathological findings mirror the capability of androgens to directly inhibit and/or counteract ER α -dependent proliferation in both normal mammary epithelium and ER α + BC preclinical models in which androgen/AR-dependent pro-apoptotic effects have been evidenced.

Here we report a novel additional mechanism, underlining the anti-proliferative role exerted by AR which involves modulation of the expression, cellular distribution and function of BAD, a pro-apoptotic Bcl-2 family protein member, whose expression is related to a significantly better disease-free survival in ER α + human BCs.

METHODS

ER α /AR-positive MCF-7, TD47D, ZR-75 BC cells; qReal-Time PCR; western blotting; IF analysis; IP assay; DAPA; ChIP Assay.

RESULTS

The expression of a panel of pro/anti-apoptotic proteins was investigated in cellular protein lysates from MCF-7 cells cultured for 1, 3 and 6 days under androgen treatment. The expression of the anti-apoptotic Bcl-2 protein, or the pro-apoptotic BID and BAX remained unchanged, while a sustained increase in the expression of the pro-apoptotic BAD was observed, reducing the Bcl-2/BAD ratio and, thus, shifting the delicate balance between inhibitors and inducers of cell death. Interestingly, androgens also induced BAD translocation into the nuclear compartment in MCF-7, T47D and ZR-75 cells. The androgen-regulated intracellular localization of BAD involved an AR/BAD physical interaction, suggesting a nuclear role for BAD upon androgen stimulation. Indeed, androgens induced both AR and BAD recruitment at a AP-1 and at a ARE site within the cyclin D1 promoter region, contributing to explain the anti-proliferative effect of androgens in breast cancer cells.

CONCLUSIONS

We defined a novel mechanism by which androgens modulate BAD expression and force its ability to act as a cell cycle inhibitor through modulation of cyclin D1 gene transcriptional activity, strengthening the protective role of androgen signaling in estrogen-responsive breast cancer.

MIR-182-5P IS UPREGULATED IN HEPATIC TISSUES FROM A DIET-INDUCED NAFLD/NASH/HCC C57BL/6J MOUSE MODEL AND MODULATES CYLD AND FOXO1 EXPRESSION.

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BACKGROUND-AIM

The non-alcoholic fatty liver disease (NAFLD) is considered a relevant liver chronic illness worldwide. Variable percentages of NAFLD cases progress from steatosis to steato-hepatitis (NASH), cirrhosis and, eventually, hepatocellular carcinoma (HCC). MiR-182-5p was found early dysregulated in a mouse model of NAFLD progression. In this study, we aimed at deepening expression levels and functional relationships between miR-182-5p and Cyld-Foxo1 in hepatic tissues from mouse models of diet-induced NAFL/NASH/HCC progression as well as to evaluate its potential role as circulating biomarker.

METHODS

MiR-182-5p expression was assessed by qRT-PCR in hepatic tissues/tumors from C57BL/6J mice model fed with high fat (HF) or low fat-high carbohydrate (LF-HC) diet and in plasma of patients with liver damage related to a condition of NAFLD progression. MiR-182-5p target genes were identified by bioinformatics analysis and validated by miRNA mimics/antagomiR transfection in HepG2 cells. Immunoblotting was performed to evaluate expression levels of two target genes, Cyld and Foxo1. Publicly available datasets were used to analyse miR-182-5p and target genes' expression in tumors from HCC patients.

RESULTS

MiR-182-5p increase, more marked in HF-fed animals, was early detected in livers as NAFLD damage progressed, and in tumor compared to peritumor normal tissues. In vitro assay on HepG2 cells confirmed that Cyld and Foxo1, both with tumor suppressor activity, were miR-182-5p target genes. According to miR-182-5p expression, decreased protein levels were observed in tumors compared to peritumor tissues. Analyses of miR-182-5p, Foxo1 and Cyld expression levels by using datasets from human HCC tumor samples showed results consistent with those from NAFLD/HCC mouse model. In contrast to hepatic tissues data, a significant decrease in plasma levels of miR-182-5p was observed in HCC compared to NASH and controls, suggesting a possible correlation between low levels of circulating miR-182-5p and progression of liver damage.

CONCLUSIONS

Our results show for the first-time miR-182-5p overexpression and Cyld-Foxo1 down-regulation, in hepatic tissues and tumors, collected from a diet-induced NAFLD/HCC mouse model. Analysis of circulating miR-182-5p expression levels also suggests its possible role as diagnostic biomarker, particularly as regard the progression of liver damage.

ROLE OF TAU/MAPT IN MODULATING DIFFERENTIATION THERAPY OF BRAIN TUMORS

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BACKGROUND-AIM

The Tau protein is expressed in neuroblastoma (NB), a pediatric extracranial solid tumor in which the younger the patient, the more the tumor retains the ability to differentiate, responding better to treatment with all-trans retinoic acid (ATRA). Tau/MAPT, under the control of kinases and phosphatases, is a multifunctional protein involved in the assembly and stabilization of microtubules that contributes to the proper transmission of axonal signal in neurons and the regulation of mitosis in proliferating cells. The aim of our study was to evaluate in neuroblastoma cells whether modulation of Tau protein phosphorylation can influence differentiation towards a neuronal phenotype, clarifying its potential role as a therapeutic target.

METHODS

As reference tumor models we used the neuroblastoma (NB) cell lines CHP-126 and SH-SY5Y. In these cells we studied in vitro the expression of Tau and the modulation of its phosphorylation status on neuronal cell differentiation.

RESULTS

Expression analysis in NB cell lines after synchronization with Nocodazole showed reversible p-Tau (Thr231) hyperphosphorylation restricted to G2/M phase, in association with a reduction in tubulin binding affinity by tau. The forced accumulation of p-Tau by okadaic acid, an inhibitor of phosphatase PP2A, was associated with aberrant mitotic spindle and cell death. Starvation by serum reduction in the culture medium, together with ATRA treatment, induced the differentiation of CHP-126 and SH-SY5Y cells, that formed branched extensions similar to those found in neurons, with cell cycle arrest in the G1/G0 phase. The spatial and functional reorganization of Tau protein observed in the neuronal phenotype as compared to the tumor phenotype suggested a role in differentiation by Tau. In fact, when we used okadaic acid in conjunction with ATRA treatment we detected a greater elongation of the neurites of the NB cells compared to ATRA treatment alone and an overall higher suppression of NB cell proliferation.

CONCLUSIONS

Our results indicate that therapeutic strategies aimed at increasing the expression of the phosphorylated form of the Tau protein may enhance the differentiation ability of neuroblastoma cells and counteract potential resistance effects associated with ATRA treatment in poorly differentiated neuroblastoma.

CIRCULATING MICRORNA SIGNATURES FOR THE DIAGNOSIS OF FAMILIAL-HEREDITARY BREAST/OVARIAN CANCER

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BACKGROUND-AIM

Circulating microRNAs (miRNAs) are emerging as very promising non-invasive biomarkers in cancer. Here, we performed a circulating miRNA profiling in hereditary-familial breast/ovarian cancer (BC/OC) cases, grouped based on the presence/absence of germline pathogenic variants in the high-penetrance susceptibility genes BRCA1/2, in order to identify differentially expressed miRNAs of potential clinical relevance as diagnostic/predictive biomarkers.

METHODS

Plasma miRNAs of a series of 32 familial BC/OC patients, including 18 BRCA1/2 positive (BRCA) and 14 BRCA1/2 negative (non-BRCA) cases, were analyzed by miRNA-sequencing using Illumina technology. Five 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. Differentially expressed miRNAs were filtered based on a log₂ fold change <-1 or >1 (down-/up-regulated miRNAs respectively), and an FDR adjusted p-value ≤ 0.05. Receiver operating characteristic (ROC) curves were built to determine miRNAs' diagnostic potential by calculating the area under the curve (AUC) with 95% confidence intervals (CI).

RESULTS

MiR-320e emerged as the most relevant miRNA able to distinguish with good accuracy (AUC 0.79; CI: 0.68-1; p-value=0.04) BC/OC cases and controls, showing down-regulation in BC/OCs compared to healthy controls. A total of 23 differentially expressed miRNAs, 12 up-regulated and 11 down-regulated, were found in non-BRCA compared to BRCA cases. Among them, 12 miRNAs were also differentially expressed between non-BRCA cases and control group, thus representing a specific miRNA expression pattern of non-BRCA patients; ROC curves showed very good diagnostic accuracy (mean AUC 0.80; p-value<0.05), suggesting that this miRNA-panel could potentially be used as diagnostic/predictive signature in hereditary-familial non-BRCA BC/OC.

CONCLUSIONS

Overall, these results suggest that the analysis of circulating miRNAs expression levels, based on the BRCA1/2 germline mutational status, could provide important information for characterizing and putatively distinguishing familial-hereditary BC/OC cases, particularly non-BRCA group, and might be considered as potentially applicable in screening and prevention programs.

RELEASE OF GLUTATHIONE TRANSFERASE OMEGA 1-1 (GSTO1-1)-LADEN EXOSOMES BY CANCER CELLS.

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BACKGROUND-AIM

Glutathione S-transferase omega-1 (GSTO1-1) is a cytosolic enzyme with uncommon reductase and thioltransferase activities involved in the modulation of cancer cell survival and inflammatory response. Studies performed in our laboratories correlated GSTO1-1 overexpression with Akt and ERK1/2 pro-survival pathways activation, JNK1 apoptotic pathway inhibition and increased resistance against cisplatin toxicity (1). More recently, a proteomic profiling of human uterine extracellular vesicles (EVs) revealed the presence of GSTO1-1 in exosomes isolated from uterine lavage of fertile women (2). Against this background, the aim of this study was to evaluate the presence of GSTO1-1 in exosomes released by cancer cells and their possible role in GSTO1-1 transport between different cell types.

METHODS

The human hepatoma HepG2 cells were incubated for 48 hours in serum free media, then exosomes were isolated by positive selection with an exosome isolation kit (Miltenyi) and analyzed by immunoblotting for the presence of GSTO1-1. Subcellular localization of GSTO1-1 was evaluated by immunogold labeling transmission electron microscopy (TEM) and immunofluorescence (IF).

RESULTS

TEM analysis revealed the presence of GSTO1-1 positive vesicles within multivesicular bodies (MVBs) of HepG2 cells. The majority of MVBs are supposed to fuse with lysosomes, but some MVBs can fuse with the plasma membrane and release vesicles in the extracellular space. Immunoblot analysis of starved HepG2 cells showed no significant variations of LC3I cleavage, a marker of autophagy, and TEM confirmed the presence of GSTO1-1 in MVBs fusing with plasma membrane. Moreover, immunoblotting of exosomes isolated from HepG2 culture media confirmed the presence of GSTO1-1-bearing vesicles. Finally, co-culture of HepG2 cells with a HeLa-derived GSTO1-1 knock-out cell line was able to induce in the latter a mild GSTO1-1 positivity and an increased resistance against cisplatin.

CONCLUSIONS

Our data suggest that GSTO1-1 positive exosomes can be released by cancer cells and transferred to recipient cells, thus possibly modulating redox reactions associated with cell survival and chemoresistance.

References.

1. Piaggi S, Carcinogenesis. 2010;31(5):804-11.
2. Rai A, Proteomics. 2021;21(13-14):e2000211.

ANTI-CANCER ACTIVITY OF THE ONCOLYTIC ADENOVIRUS DL922-947 IN ESTROGEN RECEPTOR POSITIVE BREAST CANCER

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BACKGROUND-AIM

Although estrogen receptors positivity (ER+) is a pre-requisite for anti-estrogen treatment (tamoxifen) in breast cancer (BC), a number of patients will not respond (intrinsic resistance) and others will in time fail to respond (acquired resistance).

Thus, novel therapeutic approaches are needed. Oncolytic viruses (OVs) are promising anticancer therapeutics, that selectively replicate in and kill cancer cells. In particular, the oncolytic adenovirus dl922-947 is a replicating oncolytic adenovirus bearing a 24bp deletion in E1A-Conserved Region 2, by deleting this domain viral replication can only proceed in cells with defective pRb/G1-S cell cycle checkpoint, an abnormality observed in the majority of human cancer cells. We have already demonstrated that dl922-947 is effective in treating BC both as a single agent and in combination therapies. In this study, we investigated the anticancer efficacy of dl922-947 in MCF-7 (ER+) BC cell line.

METHODS

We used MCF-7 cell line to evaluate the anticancer effects of dl922-947. Cell cytotoxicity was determined by sulforhodamine B assay 72 hours post infection (hpi). To characterize the mechanism underlying dl922-947 effects on cancer cell viability, we investigated the expression of VEGF and IL-8 48 and 72 hpi by ELISA. Furthermore, we investigated phagocytosis by human peripheral blood mononuclear cells (PBMC) exposed to the conditioned media (CM) collected after incubation of MCF-7 with dl922-947 for 6 days. Phagocytosis was evaluated by flow cytometry.

RESULTS

We addressed the anti-cancer activity of dl922-947 in MCF-7 cell line. The cytotoxic effect was accompanied by significant reduction of VEGF and increase of IL-8. IL-8 secretion is known to correlate with patient survival in luminal A breast cancer. Finally, we observed that PBMC showed enhanced phagocytosis when exposed to CM containing the virus.

CONCLUSIONS

Overall, oncolytic viral therapy might represents a possible therapeutic strategy against ER+ breast cancer; further investigations are required to address OV efficacy in treatment resistant ER+ breast cancers.

EXPLORING THE IMMUNOMODULATORY POTENTIAL OF PANCREATIC CANCER-DERIVED EXTRACELLULAR VESICLES THROUGH PROTEOMIC ANALYSIS

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BACKGROUND-AIM

Pancreatic cancer (PC) is refractory to immunotherapy and shows a highly immunosuppressive tumor microenvironment. Tumor-derived extracellular vesicles (EVs) play a role in modulating immune responses and might affect immunotherapy outcomes.

METHODS

EVs derived from Capan-2 PC cells were isolated by ultracentrifugation and characterized by atomic force microscopy, western blot (WB) and NTA sizing. Fresh PBMCs from healthy donors (HD) were treated with EVs and CD3+ lymphocytes were isolated by fluorescence-activated cell sorter. EVs and sorted CD3+ lymphocytes from EV-treated, or -untreated PBMCs were subjected to proteomic analysis. IFN- γ concentration in supernatants was measured by ELISA and CD8+PD1+ lymphocytes by flow cytometry.

RESULTS

Capan-2-derived EVs had a globular shape, an average size of 165 nm and expressed EV-specific markers by WB. Proteomic analysis of Capan-2-derived EVs identified 95 proteins connected in a single functional network ($p=1 \times 10^{-16}$) by STRING. According to IPA, 83 out of 95 identified proteins were involved in "Extracellular exosome" (FDR 3.38×10^{-63}), confirming the EV origin of the protein dataset. The highest ranked IPA downstream pathways in EVs were "Immune mediated inflammatory disease" ($p=1.84 \times 10^{-21}$) and "Leukocyte migration" ($p=3.74 \times 10^{-16}$), consistent with their potential immunomodulatory role. Proteomics of EV-treated vs. untreated CD3+ indicated "Cell viability" (z-score 5.801) and "Cell survival" (z-score 5.811) among the most activated downstream effects. Consistent with these effects, IPA analysis revealed that IFN- γ was a highly activated upstream regulator in EV-treated CD3+ lymphocytes. This result was corroborated by ELISA measurements of IFN- γ in the supernatants of EV-treated CD3+. In addition, stimulation of HD-derived PBMCs with EVs derived from two different PC cell lines increased the percentages of CD8+PD1+ lymphocytes, as compared to untreated or control EV-treated PBMCs.

CONCLUSIONS

Our proteomic and biological analyses indicate that, under the in vitro conditions tested, pancreatic cancer-derived EVs may play a role as stimulators of antitumor immunity.

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KCASH2KO MOUSE MODEL: A TOOL TO STUDY THE HEDGEHOG PATHWAY.

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BACKGROUND-AIM

KCASH2 (KCTD Containing Cullin-adaptor, Suppressor of Hedgehog 2) is a protein belonging to KCTD protein family. This protein contains a BTB/POZ domain that mediates the binding with a variety of interactors. Indeed, KCASH2 has been reported to be a suppressor of Hedgehog pathway due to its ability to form a complex with the E3 ligase Cullin3 and the histone deacetylase HDAC1, thus leading to the degradation of the latter and the subsequent silencing of the transcriptional factor GLI1. Given the importance of Hedgehog pathway signaling in organogenesis and tumorigenesis, we developed a mouse model to study in vivo the effects of KCASH2 protein absence. This KCASH2KO mouse model displays an exogenous β -galactosidase activity under the control of the KCASH2 endogenous promoter, allowing us to identify KCASH2 expression in different tissues and the effects of its lack.

METHODS

KCASH2 expression has been evaluated on frozen section of different tissues through β -galactosidase enzymatic assays, allowing us to identify the cell populations expressing KCASH2. Proliferation rate of cell populations within the tissues expressing KCASH2 has been evaluated through immunofluorescence analyses with staining anti Ki-67 and/or BrdU assays. Protein levels of downstream effectors of Hedgehog pathway, such as GLI1 and HDAC1, have been analyzed by Western Blot and RT-qPCR.

RESULTS

KCASH2 is expressed in a variety of tissues, including Central Nervous System, Testis and Colon. Absence of KCASH2 often leads to enhanced proliferation rate of the Hedgehog responding cell types within the tissues analyzed. Furthermore, morphological and functional abnormalities of the tissues involved can be observed when KCASH2 is missing. These features come along with the alteration of the physiological levels of the downstream effectors of the Hedgehog pathway.

CONCLUSIONS

Given the broad expression of KCASH2 among different tissues, it's reasonable to hypothesize the existence of a well-established role for KCASH2 to maintain the levels of Hedgehog signaling within physiological values. Given the importance of these pathway in both physiological and pathophysiological conditions, KCASH2 could potentially play an important role both in developmental and tumorigenic aspect.

TYPE 2 INNATE LYMPHOID CELLS AS NON-INVASIVE PROGNOSTIC BIOMARKERS OF PROSTATE CANCER

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BACKGROUND-AIM

Similar clinical and histologic patterns at Prostate Cancer (PCa) diagnosis may lead to variable outcomes. Biomarkers capable of improve risk stratification and identify clinically significant cancer at diagnosis represent a challenge in PCa managements. Extra-prostatic extension into periprostatic adipose tissue (PPAT) is a negative prognostic factor in PCa. PPAT include Innate Lymphoid Cells (ILCs), an high plastic immune population explaining either cancer promoting or suppressing activities in response to signals from tumor microenvironment (TME). Among ILCs subsets, ILC2 are AT-resident cells able to acquire migratory capacity. Thus, this study focused on (1) the potential pro-tumorigenic role of PPAT-resident ILC2 in PCa progression and (2) circulating ILC2s as possible non-invasive biomarker of PCa.

METHODS

PPAT biopsies were obtained from PCa patients stratified as Low Grade (LG; Gleason Score 6) or High Grade (HG; Gleason Scores 8,9 or 10). PPAT were processed to obtain protein lysates or to isolate ILCs. PBMC were obtained from blood samples from HG and LG patients. ILCs frequency and activity (i.e. activating and secreted cytokine) were measured by cytofluorimetric analysis. Statistical analyses were performed using GraphPad Prism 7.0.

RESULTS

HG-PPAT showed a significant increase of ILC2-released cytokines IL-13, IL-9 and IL-5 and of ILC2-activating IL-33 compared to LG-PPAT. Of note, an higher ILC2 frequency was observed in PPAT obtained from HG than LG PCa patients. Moreover, a significant increase of ILC2 frequency was found in HG-PBMC compared to LG-PBMC. Of note, ILC2-released IL-5 and IL-13 levels were significantly higher upon properly stimulation of HG-PMBC while not of LG-PBMC.

CONCLUSIONS

An increased activity (i.e. increased levels of IL-9, IL-5 and IL-13) accompanied by a more favorable TME (i.e. increased levels of IL-33) and by an enrichment in ILC2 subset in HG PCa patients highlighted a tumor-supporting role of AT-resident ILC2s in PCa progression. Circulating levels of ILC2s and their activation potential correlate with PCa histological grading highlighting ILC2s subset as non-invasive biomarkers for identification of clinically significant PCa at initial diagnosis.

TARGETING THE NF- κ B-DRIVEN LIPASE CES1 TO COUNTERACT METABOLIC ADAPTATION OF OSTEOSARCOMA CELLS

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BACKGROUND-AIM

Osteosarcoma (OS) is the most lethal bone tumor and is frequently associated with lung metastases. The metastatic potential of OS cells correlates with the ability of cancer cells to meet the increased energy demand required by this process, but the biology underlying OS metabolic adaptation is still unknown. NF- κ B signaling is upregulated in osteosarcoma thus sustaining cancer cell proliferation, chemotherapy resistance, and metastases formation. The toxicity of systemic NF- κ B blockade prompts characterizing NF- κ B downstream effectors as druggable targets. Recently, carboxylesterase 1 (CES1), has been identified in colorectal carcinoma (CRC) as an essential NF- κ B-regulated lipase promoting cancer-cell survival and metabolic adaptation under conditions 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. In light of the key roles of the NF- κ B pathway and the metabolic reprogramming in OS pathogenesis, we investigated whether CES1 could mediate the metabolic adaptation of OS cells in a nutrient-depleted environment.

METHODS

Public datasets of OS patients were analyzed. A panel of five OS cell lines were tested at the baseline for CES1 expression. Changes in CES1 levels, metabolic phenotype, and survival under energy stress conditions and with or without specific CES1 inhibitor were evaluated by qRT-PCR, seahorse, WB, and viability assay.

RESULTS

We analyzed CES1 expression in four OS cell lines and compared it to the non-tumor cell line h-FOB. We found that CES1 was basally expressed only in the most tumorigenic cell lines, U2 OS and MNNG/HOS. Although the strong dependency of malignant OS cells on glutamine as an energy source, U2 OS and MNNG/HOS were able to adapt to low glutamine availability. U2 OS and MNNG/HOS upregulated CES1 when cultured under glutamine limitation. Pharmacological CES1 blockade by commercially available GR-148672X inhibitor impaired bioenergetic parameters thus resulting in significant cell death of OS cell lines. The potential role of CES1 in OS progression was supported by bioinformatic preliminary data showing CES1 as a negative prognostic factor in OS.

CONCLUSIONS

These data underscore the involvement of the metabolic oncogenic axis NF- κ B/CES1 in OS tumorigenicity and suggest CES1 inhibition as a potential strategy to counteract OS metabolic adaptation.

GADD45B AS A POTENTIAL THERAPEUTIC TARGET IN OVARIAN CANCERF. Dall'Aglio¹, D. Verzella¹, M. Di Vito Nolfi¹, I. Flati¹, D. Vecchiotti¹, D. Capece¹, G. Franzoso², E. Alesse¹, F. Zazzeroni¹¹*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy*²*Department of Immunology and Inflammation, Imperial College London, W12 0NN London, United Kingdom***BACKGROUND-AIM**

Ovarian cancer (OC) is one of the most common gynecological tumors and has the highest mortality rate among all gynecological malignancies. Despite significant advances in the understanding of OC pathogenesis and treatment, the five-year survival remains about 50% and is related to the stage of the disease at diagnosis. Constitutive activation of NF- κ B occurs in more than 50% of human cancers, including OC. This aberrant NF- κ B activity drives survival, immune evasion, metastasis, and chemoresistance. Although NF- κ B is an interesting therapeutic target, none of the NF- κ B inhibitors have been approved for clinical use, due to on-target off-tumor toxicity. Therefore, research has focused on the development of new inhibitors that can regulate activators or downstream effectors of NF- κ B. GADD45B is a cancer-specific downstream effector of NF- κ B pathway that regulates the NF- κ B-mediated cancer cell survival. Thus, targeting GADD45B rather than NF- κ B itself is an alternative therapeutic strategy in Multiple Myeloma (MM) and could potentially benefit patients with other NF- κ B-driven cancers, including OC.

METHODS

Public datasets of OC patients were analyzed for GADD45B expression. OC cell lines were tested for GADD45B expression by qRT-PCR and WB. The new first-in-class GADD45B/MKK7 inhibitor, DTP3, was tested in OC cells. The efficacy of DTP3 treatment was evaluated by viability assay, WB, and FACS analyses.

RESULTS

Given the relationship between NF- κ B and GADD45B, we went to investigate the possible role of this pro-survival protein in OC. Our analysis of patient datasets demonstrated that high expression of GADD45B in OC is correlated with poor clinical outcome, suggesting a role for GADD45B in NF- κ B-driven OC pathogenesis. Congruently, DTP3 displayed a potent capacity to induce apoptosis or cell cycle arrest in GADD45B-expressing OC cell lines.

CONCLUSIONS

Our results suggest that NF- κ B/GADD45B axis mediates OC-cell survival and can be targeted with DTP3 to reduce OC cell viability, thereby providing a strong rationale for developing this new anti-GADD45B agent for treating OC patients.

RSV AND SARS-COV-2 CO-INFECTION: AN IN VITRO TOOL TO MONITOR PATHOGENIC MECHANISMS

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BACKGROUND-AIM

Co-infections are common and can seriously affect the course of each infection from its own natural history. Co-infecting pathogens with an analogous tropism, such as RSV and SARS-CoV-2, may antagonize or facilitate each other modulating host disease outcomes and microbe transmission relative to a single infection. Clinically, a severe phenotype has been reported in children with RSV/SARS-CoV-2 co-infections. However, experimental models to study the molecular and immunological dynamics of co-infections are extremely limited. Herein, we propose an in vitro co-infection model to assess RSV/SARS-CoV-2 immune and viral evolution.

METHODS

A549-ACE2 expressing cells were co-infected with RSV and SARS-CoV-2 (MOI=0.01 each). The replication rate of each virus was determined at 24, 48 and 72 hours post-infection (hpi) by Droplet Digital PCR (ddPCR), immune-fluorescent (IF) and transmission electron microscopy (TEM) analyses. Secretome analyses (17 Multiplex Cytokine ELISA) on cell culture supernatants and anti-viral/immune gene expression (RT-qPCR) were assessed as well. All the experiments were performed in the BSL3 facility.

RESULTS

The RSV/SARS-CoV-2 co-infection was characterized by a significant increase in the replication rate of RSV (co-infection vs single RSV $p < 0.01$) (Figure 2). Checking the expression of the main RSV receptors, a significant increase in ICAM1 was observed (co-infection vs single RSV: $p < 0.0001$) (co-infection vs single SARS-CoV-2: $p < 0.0001$). This was accompanied by a significant rise in the expression of IFN β and the main Interferon Stimulated Genes (ISG), along with pro-inflammatory genes. The secretome analysis revealed an enhanced inflammation in the co-infected condition as well, with increased levels of the main pro-inflammatory cytokines compared to single infections.

CONCLUSIONS

The RSV/SARS-CoV-2 co-infection model displays a unique and specific viral and molecular fingerprint. In particular, the co-infected condition is characterized by an increased replication rate of RSV, together with an enhanced pro-inflammatory profile, giving clues of augmented severity upon RSV infection in the context of a concomitant SARS-CoV-2 co-infection. The in vitro co-infection model may represent an attractive cost/effective approach to mimic both viral dynamics and host immune responses, providing readily-measurable targets predictive of co-infection progression.

DENDRITIC CELL CHARACTERIZATION AND T CELL POLARIZATION AFTER INFECTION WITH LEISHMANIA TARENTOLAE ENGINEERED FOR THE EXPRESSION OF THE SARS-COV-2 SPIKE PROTEIN FOR USE AS A VACCINE VEHICLE

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BACKGROUND-AIM

Leishmania tarentolae is a non-pathogenic protozoa, extensively studied as biofactory of protein. At present it is investigated as vaccine vehicle or candidate vaccine. This type of protozoa is able to target antigen presenting cells (APC), but the immune response which it induces has not yet been characterized.

METHODS

DCs were derived from human monocytes and exposed to live *L. tarentolae*, using both the non-engineered P10 strain and the same strain engineered for expression of the spike protein from SARS-CoV-2. Then DCs were cultured with autologous lymphocytes to mime antigen presentation. Parasite internalization in the DCs (Giemsa staining), DC activation (Flowcytometry), and T cell immune polarization (PCR Array) were analyzed.

RESULTS

DCs effectively internalized protozoans from both strains resulting in a full pattern of maturation, in terms of MHC class II and costimulatory molecules expression (CD80, CD83). In addition, *Leishmania* from both strains induced a Th1-like polarization profile, characterized by TNF- α , IL-6, IL-9, IFN- γ , MCP-1 production. Moreover, IL-12p70 release was higher in Lt-spike-stimulated DC supernatants while MIP and IP-10 secretion was enhanced following Lt-wt stimulation. In T-DC co-cultures, IFN- γ and IL-10 secretion were up- and down-regulated, respectively, suggesting a TH1 polarization outline.

CONCLUSIONS

Our data suggest that *L. tarentolae* behaves as a vaccine vehicle able to trigger DC activation and Th1 differentiation. Moreover, *L. tarentolae* could be used for the production and delivery of antigens in vaccination and could be associated with immune-modulating molecules to trigger the most efficient immune response.

EVALUATION OF 3D BIOPRINTING FOR TESTING SRC-TARGETED DRUGS IN CANCER MODELS

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BACKGROUND-AIM

3D tumor cell models promise to add more robust predictive data to the in vitro study. Recent advances in 3D printing techniques and biomaterials have offered new tools in developing 3D tissue cultures. 3D bioprinting is a new approach that offers high reproducibility and precise control of printed 3D structures. Our work aimed at developing new 3D tumor models and at verifying their potential role in the prediction of antitumor efficacy of Src inhibitors.

METHODS

In our study, we compared the sensitivity to Src inhibitors in two glioblastoma cell lines, U87 and U251, cultured in 3D bioprinting model with 3D spheroid model and 2D models. The hydrogel used in bioprinting was a combination of sodium alginate and gelatin. We evaluated the activity of dasatinib and of two novel [3,4-d]pyrazole pyrimidine inhibitors.

RESULTS

U87 and U251 cells were able to proliferate in 3D alginate/gelatin bioprinted structures for two weeks, forming spheroid aggregates, and maintaining high levels of Src activation. Bioprinted cells were significantly more resistant to Src inhibitors toxicity than the corresponding cells cultured in monolayers showing IC50s similar to those observed in the spheroid models. Recovery and analysis of the cells from the bioprinted cultures showed a reduced ability of compounds to inhibit Src activation within 3D structures.

CONCLUSIONS

The 3D bioprinted model utilizing alginate/gelatin hydrogel represent a suitable model in drug screening when spheroid growth is required, offering advantages in feasibility and reproducibility compared to the classical spheroids model.

EVALUATION IN VITRO OF THE POTENTIAL ANTI-FIBROTIC EFFECT OF CURRENT TREATMENTS FOR INTESTINAL BOWEL DISEASE.

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BACKGROUND-AIM

Intestinal fibrosis is a common and severe complication of intestinal bowel disease (IBD). TGF- β 1 plays a central role in the fibrotic process by inducing myofibroblast activation and excessive extracellular matrix (ECM) protein deposition. The fibrotic process requires the involvement of the activated myofibroblasts and the epithelial-mesenchymal transition (EMT). The current treatment for IBD is based on anti-inflammatory drugs (mesalamine, steroids, immunosuppressant, and biological), which, even if they show clinical effects on the acute phase of the IBD, do not prevent or counteract the induction of the fibrotic process. Of note, very few studies have evaluated the potential anti-fibrotic effects of these drugs. In the present work we investigated the direct impact of drugs currently used in IBD on in vitro cell models of activated myofibroblasts and EMT.

METHODS

Human intestinal fibroblast cell line (CCD-18Co) was exposed to TGF- β 1 for 48h to induce the differentiation to activated myofibroblasts, while the intestinal epithelial cell line (Caco-2) for 4 days to establish the EMT in presence or absence of mesalamine, azathioprine, methotrexate, methylprednisolone, prednisone, budesonide, infliximab, and adalimumab. The cell viability and the levels of collagen I, pSMAD2/3, α -SMA, and occludin were assessed by MTT assay and western blotting, respectively.

RESULTS

All drugs used did not influence cell viability in both models, thus excluding toxic effects. Among tested drugs, only methylprednisolone and adalimumab induced a decrease of collagen I and alpha-SMA on activated myofibroblasts, counteracting the effects of TGF- β 1. In the EMT model, a reduced alpha-SMA expression and an increase of occludin were evident just after methylprednisolone and budesonide treatment, counteracting, in part, the TGF- β 1-induced transition.

CONCLUSIONS

Our results show that only steroids and adalimumab could exert a mild or moderate anti-fibrotic effect, thus supporting that the pharmacological anti-inflammatory action is insufficient to counteract the fibrotic phenotype.

IN VIVO EFFECT OF ORAL DAILY INTAKE OF THE ENDOCRINE DISRUPTOR BISPHENOL-A (BPA) IN MICE TRANSGENIC FOR THE NEU ONCOGENE, WHICH SPONTANEOUSLY DEVELOP MAMMARY TUMORS

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BACKGROUND-AIM

Breast cancer represents the most prevalent cancer worldwide. Environmental pollutants can increase cancer risk and modulate cancer aggressiveness by acting as endocrine disruptors (EDs). In this context, Bisphenol-A (BPA), a compound used in the manufacture of plastic and plastic-derived products, displays ED properties due to its ability to bind estrogen receptors (ER) and act as a xenoestrogen. Indeed, its role in the intraductal hyperplasia of rats' mammary glands and in the increase in mammary gland density in non-human primates is well demonstrated. In the present study, we evaluated BPA contribution to cancerogenesis in mice transgenic for the neu oncogene (BALB-neuT).

METHODS

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

RESULTS

BPA increased the number of tumors per mouse and reduced both disease-free survival and overall animal survival. BPA significantly increased the expression of PR at 6, 11, and 30 weeks, of Ki67 at 11 and 30 weeks, and of phospho-AKT at 30 weeks in cancer cells. CD31⁺ endothelial cells were, as well, increased in BPA-treated animals at 30 weeks. Studies of the tumor-infiltrating immune cells showed that CD4⁺ cells were increased at 6, 11, and 30 weeks in mammary tissues of BPA-treated mice, paralleled by the concurrent increase of Foxp3⁺ and PD-1⁺ cells at 30 weeks.

CONCLUSIONS

Oral daily intake of BPA, at currently authorized concentrations, accelerated neoplastic transformation and affected infiltrating tumor cells in the BALB-neuT mouse model. The revision of the tolerable daily intake of BPA is therefore of paramount importance in order to reduce the carcinogenic risk associated with exposure to this chemical compound.

IDENTIFICATION OF A CROSS-TALK BETWEEN THE MEK5/ERK5 AND THE HEDGEHOG/GLI PATHWAYS IN MELANOMA

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BACKGROUND-AIM

Malignant melanoma is among the most aggressive cancers and its incidence is increasing worldwide. We have reported that the Mitogen-Activated Protein kinase ERK5 promotes melanoma growth in vitro and in vivo. Additionally, we have recently reported that ERK5 is required for the Hedgehog/GLI (HH/GLI)-dependent melanoma cell proliferation and that GLI1 positively regulates the expression of ERK5. Since the HH/GLI pathway may be activated in a non-canonical way by the MAPK ERK1/2, we explored whether ERK5 positively regulates the HH/GLI signaling.

METHODS

BRAFV600E-mutated (A375 and Sk-Mel-5) and wild-type BRAF (SSM2c) melanoma cell lines and murine NIH/3T3 fibroblasts were silenced for ERK5 using ERK5-targeting shRNAs or treated with a non-targeting shRNA (shNT) as a negative control. Luciferase assay using the GLI-binding site Luciferase reporter was performed to evaluate GLI transcriptional activity. A constitutively active form of MEK5 (MEK5DD) was used to induce activation of endogenous ERK5 or overexpressed ERK5. Chemicals (small molecule inhibitors) used were: the ERK5 inhibitors JWG-071 and AX-15836; MEK5 inhibitors GW284543 and BIX 02189; GLI1/2 inhibitor GANT61; SAG, an HH-GLI pathway activator. Activation of HH/GLI pathway was obtained by PATCH1 silencing. 3D spheroid assays were performed in SSM2c and A375 cells treated with GANT61 in combination with the MEK5 inhibitors.

RESULTS

Treatment with ERK5 inhibitors reduced transcriptional activity of endogenous HH-GLI pathway in a dose dependent manner in NIH/3T3 cells. This effect was recapitulated upon ERK5 genetic inhibition, which determined a reduction of GLI1 and GLI2 proteins. MEK5DD overexpression, which determined ERK5 activation, further increased transcriptional activity of SAG-induced HH/GLI, while silencing of endogenous ERK5 reverted this effect. These results confirmed that ERK5 positively regulates the HH/GLI signaling. Consistently, MEK5DD overexpression increased GLI1 and GLI2 protein levels. In melanoma cells, genetic and pharmacological ERK5 inhibition similarly inhibited the expression and activity of GLI proteins. Interestingly, the combination of GANT61 with MEK5 inhibitors was more effective than single treatments in reducing the volume of melanoma spheroids.

CONCLUSIONS

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

FROM 2D TO 3D STUDIES: NEW INSIGHTS AND THERAPEUTIC APPROACH INTO THE GLAUCOMA PATHOGENESIS

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BACKGROUND-AIM

Glaucoma is an optic neuropathy characterized by a progressive degeneration of retinal ganglion cells (RGCs). It is known that the major risk of neuronal injury is an elevated intraocular pressure (IOP) but molecular mechanisms involved in cellular degeneration and in stress response are poorly understood. 2D cultures in this contest show many limitations including affected cell polarity and absence of an extracellular matrix (ECM). 3D bioprinting can be a useful approach to overcome these challenges allowing the creation of 3D structures composed by living cells encapsulated in specific bioinks mimicking native ECM. The aim of this study is to determine the potential therapeutic efficacy of rhNGF in glaucoma both in vitro and in vivo

METHODS

Glaucoma has been simulated in vitro using millifluidic bioreactor. 3D bioprinting have been used to develop a hybrid 3D model constituted by bioink-embedded Rat Retinal Müller Cells (rMC1) on top of ARPE19 monolayer thus mimicking neuroretinal and retinal pigment epithelium (RPE) layers, respectively. These models were analyzed by Western Blot (WB) and PrestoBlue assay. In vivo study was carried out by using DBA/2J mice. Electroretinogram and Optometry test has been performed before and 4 weeks after compound administration. IHC and WB on enucleated eye samples were used for molecular studies

RESULTS

2D cultures showed that rMC1 and ARPE19 respond to IOP by modulating Akt, MAPK and NF- κ B pathways. In addition, IOP affects rMC1 and ARPE19 proliferation. Increased IOP induces p27 expression in ARPE19 and activates Müller glia cells. RhNGF promotes ARPE19 proliferation and triggers mTOR signaling in both cell lines. A 3D retinal model was analyzed under flow and hydrostatic pressure via LiveFlow system. rhNGF treatment appears to be effective in modulating proliferation and cell metabolism via mTOR/AMPK α signaling. In vivo study highlighted a significant increase of the visual acuity and scotopic a-wave amplitude at 0.1, 1 and 10 cd s/m² was observed in the glaucomatous animals treated with rhNGF compared to the vehicle at 32 weeks old. RhNGF administration in non-glaucomatous animals allowed to delay the glaucoma setup by reducing visual and electrophysiological impairment

CONCLUSIONS

Our data demonstrate the predictivity of proposed 3D models and confirm a potential efficacy of the rhNGF in vitro as well as on the visual acuity and electrophysiological impairment of retina induced by glaucoma when administered by intravitreal route in DBA/2J mice

ANGIOTENSIN II THROUGH PI3K/AKT AND HEDGEHOG CROSSTALK PROMOTES GLIOBLASTOMA STEMNESS

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BACKGROUND-AIM

Introduction: Glioblastoma Multiforme (GBM) is the most common and aggressive primary brain tumor. The prognosis is very poor, with most patients surviving only 12 to 15 months after diagnosis, due to resistance to treatment, that includes surgery, radiation therapy, and chemotherapy. GBM is characterized by a wide phenotypic and cellular heterogeneity, maintained by stem-like cells, known as glioma stem cells (GSCs). Notably, GSCs contribute to tumorigenesis, invasion, recurrence, and resistance to therapy, due to their capacity to self-renewal, regulated by several pathways, including Hedgehog (Hh) signaling. Furthermore, it has been evidenced the role of renin-angiotensin system and its receptors in the reorganization of cellular matrix and their involvement in pro-tumoral function, suggesting them as potential therapeutic targets. Moreover, we demonstrated that ANGII/AGTR1 signaling enhances local estrogen production in GBM through the upregulation of aromatase gene expression, sustaining tumor progression. The aim of this study was to investigate how anti-estrogens drugs may work against stimulatory effects of ANGII in maintenance of GBM stemness.

METHODS

Methods: U87MG cell line was treated with ANGII(5#M), anti-estrogen ICI-182,780 (ICI)(1#M) and LY294002(10#M) alone or in combination. Transcriptomic analyses were performed to identify genes involved in tumor growth and progression. qRT-PCR and western blotting assay were carried out to analyze signaling trasductional pathways. Neurosphere formation (NF) was evaluated to investigate glioblastoma stemness.

RESULTS

Results: ANGII treatment promotes the crosstalk between PI3K/Akt and Hh through non genomic activation of estrogen signaling. This results in the enhanced phosphorylation of Akt and expression of GLI1, a nuclear mediator of Hh pathway, sustaining GBM stemness. All this raises by the evidence that in the presence of either inhibitors of PI3K/ Akt or the pure anti-estrogen ICI the up regulatory effect on GLI1 expression and stemness, revealed by a reduction of NF, are no longer noticeable.

CONCLUSIONS

Conclusions: Based on these findings, it is reasonable to repurpose existing drugs, such as ICI, which may potentially offer several advantages including lower costs, faster development timelines and improve GBM patient outcomes.

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

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BACKGROUND-AIM

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

METHODS

Here, we treated lymphatic endothelial cells (LECs) in vitro with lymphangiogenic stimuli in the absence or in the presence of recombinant PTX3 and we verified the effect of exogenous PTX3 on LEC activation, in terms of proliferation, migration and sprout formation. The activation of FGF/FGFR system was investigated through western blot analysis. Furthermore, in vivo matrigel plug assay and lymphatic dissemination of melanoma cells were performed in mice characterized by lymphatic expression of PTX3. The effect of lymphangiogenic stimuli and melanoma-derived factor on PTX3 expression in LECs was assessed in vitro through real time PCR and western blot. RNAscope analysis was performed on human melanoma samples to investigate lymphatic expression of PTX3 in the primary tumor.

RESULTS

This preliminary work sheds light on the regulatory role of PTX3 in lymphangiogenesis and in melanoma lymphogenous dissemination. Indeed, treatment with recombinant PTX3 reduces LEC activation by inhibiting FGF/FGFR signaling. Furthermore, we observed that lymphatic expression of PTX3 hampers lymphangiogenesis in vivo and significantly reduces the metastatic spreading of melanoma cells to the draining LN. Moreover, we observed that lymphangiogenic and melanoma-derived factors downregulate PTX3 expression in LECs. Accordingly, PTX3 downregulation occurs in lymphatic vessels of primary human melanoma specimens when compared to normal skin.

CONCLUSIONS

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

EFFICACY OF ONCOLYTIC ADENOVIRUS dl922-947 IN TRIPLE-NEGATIVE BREAST CANCER CELL LINES

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is characterized by poor prognosis and limited treatment options. Novel therapeutic approaches are urgently required and among new treatments, the oncolytic virotherapy might represent a promising opportunity. dl922-947 is a second-generation selective replicating oncolytic adenovirus bearing a 24bp deletion in E1A-Conserved Region 2. This deletion restricts viral replication to cells with defective pRb/G1-S cell cycle checkpoint, a common abnormality in most human cancer cells. In this study we aimed to investigate the anticancer efficacy of dl922-947 in TNBC cell lines.

METHODS

We used two TNBC cell lines: MDA-MB-231 and MDA-MB-468. Cell cytotoxicity was determined by sulforhodamine B assay. To characterize the mechanism underlying dl922-947 effects on cancer cell viability, we assessed its capacity to induce apoptosis and Immunogenic Cell Death (ICD), characterized by three markers (Calreticulin, HMGB1 and ATP). Apoptosis, calreticulin expression and HMGB1 secretion were determined by flow cytometry, whereas ATP content was assessed by a luminescence-based bioassay. Furthermore, we investigated the modulation of cytokines known for their role in breast cancer growth and spread (IL-6, IL-8 and VEGF) by ELISA on cell-free supernatant. Finally, we evaluated the IL-6 activated cGAS-STING-STAT3 pathway by Western blot.

RESULTS

We evaluated dl922-947 induced cytotoxicity and we observed higher efficacy in MDA-MB-231 cell line. Consequently, we focused our further assays on MDA-MB-231 cells. dl922-947 infection induces apoptosis and modulates ICD markers, including calreticulin surface expression, HMGB1 and ATP release. Furthermore, dl922-947 reduces IL-6, IL-8 and VEGF secretion and modulate cGAS-STING-STAT3 pathway that is highly activated in TNBC cells.

CONCLUSIONS

Our data suggest that dl922-947 induces anticancer effects in TNBC cells, possibly associated with an anti-tumor immune response. Overall, our study highlights the potential of oncolytic virotherapy as a promising therapeutic approach against TNBC.

APPLICATION OF MOLECULAR IMAGING AS POTENTIAL EARLY BIOMARKER FOR TRIPLE-NEGATIVE AND ESTROGEN RECEPTOR POSITIVE BREAST CANCER MODELS

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BACKGROUND-AIM

Breast cancer (BC) represents one of the most common tumors diagnosed in women worldwide with high mortality rate and it is characterized by a remarkable degree of morphological and molecular heterogeneity. A hallmark of human cancer, including BC, is the metabolic reprogramming towards glycolysis upregulation becoming an intriguing therapeutic target for metabolic drugs, such as metformin. A synergistic activity against different tumor types has been provided by the administration of metformin in combination with the anti-hypertensive syrosingopine, a dual inhibitor of the monocarboxylate transporters MCT1 and MCT4, increasing intracellular lactate levels and thus limiting glycolysis. We aimed to in vivo investigate the early response to treatment, based on metformin and/or syrosingopine administration, evaluating the [18F]FDG and [18F]FLT as potential PET/CT biomarkers.

METHODS

Balb/c female mice were subcutaneously inoculated with triple negative murine BC cell line (TS/A) and a metastatic ER+ murine BC cell line (4T1). Xenografts models were divided into six treatment groups: vehicle, cisplatin, metformin, syrosingopine or cisplatin plus metformin and metformin plus syrosingopine. The response to treatment was monitored by caliper measurements, for tumor volume and PET/CT studies. Molecular biomarkers of glucose metabolism and tumor invasiveness were analyzed by means of Real-Time quantitative PCR (RT-qPCR) and immunohistochemistry (IHC) analyses.

RESULTS

A significant tumor growth inhibition (%TGI) has been observed after metformin plus syrosingopine administration, confirming a synergistic effect after seven and ten days of treatment. Compared to ER+ model (TS/A), this drug combination showed the highest efficacy on the TNBC xenograft mice (4T1) via the lowering of lactate transporter MCT4 levels, as validated by RT-qPCR and IHC analyses. Moreover, PET imaging revealed a significant reduction of [18F]FLT tumor uptake metformin plus syrosingopine-treated groups, whereas [18F]FDG uptake increased in all experimental conditions.

CONCLUSIONS

[18F]FLT radiotracer could be potentially used as a biomarker for the early prediction of therapy response in both evaluated xenografts models but further investigations are required to better clarify the mechanisms underlying the synergistic effect of syrosingopine plus metformin in these BC models.

MACROPHAGE ACTIVATION DUE TO TLR4- AND TLR7/8 AGONISTS IS LIMITED BY NRF2/HO-1 STIMULATION

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BACKGROUND-AIM

The transcription factor NRF2 controls a plethora of genes with pro-surviving activity, among which heme oxygenase 1 (HO-1) has been recognized to play a potent anti-inflammatory activity. Macrophages trigger inflammation by recognizing pathogens and tissue damage using toll like receptors (TLRs). In particular, TLR4 and TLR7/8 have been proved to have downstream signal cascades highly interrelated, involving both NF- κ B and interferon regulatory factor 5 (IRF5) transcription factors and leading to pro-inflammatory cytokine release (e.g. TNF- α). Interestingly, IRF5 is dysregulated in autoimmune and cardiovascular diseases, and in neuroinflammation. Our work aims at understanding the involvement of NRF2/HO-1 in the regulation of IRF5.

METHODS

Murine macrophage-like cells RAW 264.7 and human promyelocytic cell line THP-1 (undifferentiated and PMA-treated) have been exposed to 100ng/ml LPS (TLR4 agonist) and to different doses of R848 (TLR7/8 agonist). NRF2/HO-1 was induced by cell exposure to sulforaphane (SFN). NF- κ B activation was measured by WB analysis of p-I κ B α . IRF5 was evaluated by WB and its activation was measured by checking its dimerization. TNF- α induction was measured by qPCR and TNF- α secretion through ELISA. HO-1 induction was evaluated by qPCR and WB. HO-1 activity was evaluated measuring bilirubin generation. HO-1 activity was inhibited using 10 μ M tin mesoporphyrin IX (SnMP).

RESULTS

RAW 264.7 cells exposed to LPS showed an early NF- κ B activation followed by TNF- α induction at 6 and 24h. At 24h, IRF5 and HO-1 protein levels were also increased. SnMP further induced TNF- α mRNA expression and increased IRF5 levels after 24h of LPS treatment. Moreover, R848 induced IRF5 dimerization and TNF- α expression. Co-treatment with SFN increased HO-1 expression, reduced IRF5 activation and decreased TNF- α induction. THP-1 cells differentiated with PMA showed the same results on TNF- α mRNA expression when treated with R848 and SFN. In addition, undifferentiated THP-1 exposed to increasing doses of SFN showed a progressive reduction of IRF5 expression while increased the HO-1 expression.

CONCLUSIONS

We show that TLR4 stimulation activates HO-1 that limits pro-inflammatory activation reducing IRF5 expression. NRF2/HO-1 stimulation prevents IRF5 activation downstream TLR7/8 and reduces the basal expression of IRF5. Our data contribute to shed a light on molecular mechanisms underlying the dysregulation of TLR4- and TLR7/8-dependent responses.

THE MRN COMPLEX REGULATES PRIMARY CILIOGENESIS TO SUSTAIN NEURONAL PROGENITOR PROLIFERATION

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BACKGROUND-AIM

The MRN (MRE11/RAD50/NBS1) complex is essential for the activation of the DNA Damage Response (DDR). Hypomorphic mutations in each component of the complex cause DDR defective syndromes characterized by immunodeficiency, predisposition to cancer and microcephaly. Interestingly, microcephaly is a common outcome of mutations in proteins operating at the centrosome or at the primary cilium (PC), an antenna-like organelle which is essential for the regulation of specific neurodevelopmental signaling pathways, like the Sonic Hedgehog (SHH) one. We recently showed that Central Nervous System (CNS)-restricted NBS1-KO in mice completely abolishes SHH-driven medulloblastoma (MB) and that it correlates with SHH pathway downregulation. Since MRN proteins localize at the centrosome and control its duplication, and MRE11 mutation leads to a Nephronophthisis-related ciliopathy, we speculated that, in addition to its canonical role in the DDR, the MRN complex may regulate the SHH pathway, impacting on cerebellar development and tumorigenesis, by controlling the PC in the Granule Cell Progenitors (GCPs), the most abundant population in the cerebellum and from which SHH-MB arises.

METHODS

We manipulated both human cells and primary GCPs in vitro, and examined RNA and protein expression by Microfluidic CARD and Western Blot analyses, respectively. We also generated and analyzed new mice models with GCPs-restricted NBS1-KO in both WT and SHH-MB prone backgrounds. Finally, we evaluated BB/PC morphology by confocal microscopy.

RESULTS

We found that MRN proteins localize at the Basal Body (BB). The depletion of NBS1 and MRE11, but not of RAD50, induces an elongated and dysmorphic PC, as well as MRE11 pharmacological inhibition via mirin. Importantly, PC phenotypes induced by NBS1-KO are dependent on p53 and reproduced in immortalized dermal fibroblasts obtained from a NBS patient. Moreover, GCP-restricted NBS1-KO, reducing proliferation, impairs and abrogates SHH-dependent cerebellar development and MB insurgence, respectively. Finally, NBS1 depletion inhibits SHH pathway, in vivo, ex vivo and in vitro models.

CONCLUSIONS

Our data indicate that the MRN complex exerts a previously undisclosed function in the regulation of the PC/SHH pathway that eventually impacts on cerebellar development and tumorigenesis.

DISSECTING THE CHARACTERISTICS OF "UNCONVENTIONAL" CIRCULATING TUMOR CELLS

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BACKGROUND-AIM

Circulating tumor cells (CTCs) were first described 150 years ago. The so-called "classical" CTC populations (EpCAM+/CK+/CD45-) have been fully characterized and proposed as the most representative CTC subset, with clinical relevance. Nonetheless, other "atypical" or "unconventional" CTCs have also been identified and could have a critical role in metastasis formation. Unconventional CTCs are defined as CTCs that display both epithelial and mesenchymal markers, or both cancer and immune markers, also known as hybrid cancer-immune cells.

METHODS

We obtained evidence of the presence of unconventional CTCs in the circulation of lung cancer and cancer of unknown primary (CUP) patients, using CellSearch technology (Menarini Silicon Biosystems).

RESULTS

Transcriptome analysis of CTC-derived cell lines supported the presence of subpopulations expressing immune-related markers. Moreover, short-term cultures of conventional and unconventional CTCs obtained from pleural effusions revealed a marked difference in growth capabilities of the two CTC subtypes.

CONCLUSIONS

We believe that a deeper knowledge on unconventional CTCs is instrumental to elucidate their role in cancer progression.

ROLE OF INHERITED PREDISPOSITION AND INTESTINAL MICROBIOTA IN COLORECTAL CARCINOGENESIS

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BACKGROUND-AIM

Colorectal cancer (CRC) is one of the most common tumors and has a multifactorial etiology, combining genetic, environmental and life-style factors. Familiarity is found in 30% of the patients, but a monogenic inherited cancer syndrome is diagnosed in only 6% of cases. Increasing data show that gut microbiota may act as a risk factor for CRC, but a small number of studies focused on patients with inherited cancer predisposition. Since contrasting effects of butyrate (a metabolite produced by some bacteria) were reported in mice that mimic genetic syndromes or sporadic CRC, we hypothesized a different effect of gut microbiota on tumor development in different genetic backgrounds.

Our aim was to characterize the gut microbiota and the genetic background of patients with colon polyps to compare the microbiota of sporadic cases and patients with inherited risk factors.

METHODS

We recruited 179 patients with colon polyps and collected peripheral blood, mucosa-associated microbiota (MAM), information on family history of cancer, nutritional habits (validated EPIC questionnaire). Germline DNA from peripheral blood was analyzed by targeted-NGS using a custom panel of 107 genes involved in inherited cancer syndromes. MAM was identified by 16S ribosomal DNA sequencing on 120 out of 179 patients.

RESULTS

Twenty-one patients (11.7%) carried germline pathogenic variants (PVs) in APC, BLM, BMPR1A, BRCA1, BRIP1, CDKN2A, FANCG, FANCM, GALNT12, MLH1, MSH6, MUTYH, NTHL1, RAD50, RAD51C, SBDS, TSC2, VHL and could be considered as affected by cancer predisposition syndromes. We identified different MAM signatures in mutated vs sporadic patients: Akkermansia genus was enriched in mutated cases, while Unclassified Bifidobacteriaceae and Bifidobacterium genera in the sporadic group ($p < 0.05$). We excluded from the comparison patients with family history of cancer, since they could carry mutations not identified by our approach. No statistically significant difference in body mass index was identified between the two groups.

CONCLUSIONS

These preliminary data support our hypothesis that different genetic backgrounds may be associated to distinct bacterial risk factors. This may suggest new preventative options for patients carrying an inherited cancer syndrome. This project was supported by AIRC-25886.

PERI-RENAL ADIPOCYTE-FACTORS DRIVE CLEAR CELL RENAL CELL CARCINOMA METASTATIZATION ENHANCING CELL VIABILITY AND MOTILITY

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BACKGROUND-AIM

Clear cell Renal Cell Carcinoma (ccRCC) is the most common and lethal subtype of kidney cancer. Despite nephrectomy with curative intent, about 30% of patients eventually develop metastases. Epidemiologic investigation showed that Peri-Renal Adipose Tissue (PRAT) thickness is significantly associated with a poor prognosis. In this scenario, the adipokines released by PRAT could drive cancer spread increasing cell migration and survival. However, the molecular mechanisms involved in this cross-talk are still unknown.

METHODS

Adipose-derived mesenchymal stem cells (Ad-MSCs) were obtained from biopsy of PRAT from ccRCC patients and from healthy kidney donors undergoing radical nephrectomy. Ad-MSC were differentiated in mature adipocytes and then serum starved to obtain conditioned media (Ad-CM). Two ccRCC cancer cell line, ACHN and 786-O, were used to assess the effect of Ad-CM on cell viability and migration. Protein samples were analyzed by Western blot with pFAK antibody. GAPDH antibody was used for normalization.

RESULTS

The treatment for 24h with Ad-CM obtained from of ccRCC patient's adipose tissue (ccRCC Ad-CM) induced the increase of cell viability in ACHN, while no effects was observed with Ad-CM deriving from Healthy Donors (HD Ad-CM). The same treatment had no effect in 786-O cell viability. Next, we assessed the effect of Ad-CM on cell motility in 786-O and ACHN, the treatment for 6h with ccRCC Ad-CM increased cell motility and pFAK expression in both cell lines. No effect was observed treating 786-O and ACHN with HD-Ad-CM.

CONCLUSIONS

Increased viability and migratory capacity are a hallmark of an unfavorable prognosis. Our result demonstrated that tumor surrounding adipocytes promote migration through a pFAK-dependent mechanism highlighting the relevant role played by PRAT surrounding the tumor on ccRCC clinical outcome and offering new chances to develop personalized treatment for patients with advanced ccRCC.

TXNIP EXPRESSION IS TRANSCRIPTIONALLY REGULATED BY FOXO3A IN TAMOXIFEN-SENSITIVE AND RESISTANT BREAST CANCER CELLS

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BACKGROUND-AIM

Endocrine therapy resistance is a major challenge in the treatment of estrogen receptor positive (ER+) breast cancer (BC) patients. The Forkhead box class O 3 (FoxO3a) transcription factor acts as an oncosuppressor that regulates several target genes involved in cell cycle arrest, apoptosis, and metabolism. Among the others, FoxO3a also seems to control the transcription of another interesting tumor suppressor gene, Thioredoxin interacting protein (TXNIP), whose levels are often low in different tumors, as BC, and associate with poor prognosis. Since FoxO3a is a positive prognostic marker in ER+ BCs and its downregulation may confer tamoxifen (Tam) resistance, the aim of the present work was to assess if TXNIP might be crucial in mediating FoxO3a effects in ER+ BC cells (BCCs) and in their Tam-resistant (TamR) counterparts.

METHODS

As experimental models we used ER+ MCF-7 and the deriving TamR BCCs, generated after long-term exposure to Tam. TamR/TetOn-AAA BCCs, stably transfected with a plasmid bearing a cDNA encoding a constitutively active FoxO3a gene, whose expression is induced by Doxycycline, and the relative control vector TamR/TetOn-V BCCs were also employed. The interplay between FoxO3a and TXNIP was evaluated by Western blot (WB), qRT-PCR, siRNA approaches, and Chromatin Immunoprecipitation (ChIP) assay.

RESULTS

Transient over-expression of FoxO3a in both MCF-7 and TamR BCCs was able to increase TXNIP mRNA and protein levels. Similar results were observed in TamR/TetOn-AAA BCCs compared to control TamR/TetOn-V cells. As expected, TXNIP silencing led to a decrease in TXNIP expression in our cell models, confirming that TXNIP gene is regulated by FoxO3a. Interestingly, ChIP experiments evidenced a significant recruitment of FoxO3a on the TXNIP promoter region containing a Forkhead Responsive Element (FHRE) motif in MCF-7 and TamR cells and in inducible stable cell lines, further highlighting FoxO3a involvement in TXNIP gene regulation.

CONCLUSIONS

Our data unveil the existence of a FoxO3a-dependent transcriptional regulation of the oncosuppressor TXNIP in ER+ BCCs and in TamR cell models. Ongoing investigations will elucidate FoxO3a/TXNIP interplay in both predicting and overcoming the development of drug resistance.

A PERSPECTIVE ON GOLD NANOPARTICLES APPLICATION IN THE TREATMENT OF GASTRIC CANCER

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BACKGROUND-AIM

Gastric cancer (GC) remains one of the most common cancers worldwide being the fourth malignancy for incidence and the third for mortality.

Although surgical resection with D2 lymphadenectomy remains the mainstay of curative therapy for GC, the prognosis of patients remains overall poor since in early stages GC is often asymptomatic and most of patients present an advanced and inoperable disease at diagnosis dropping down 5-year survival rates to 10% for patients with distant-stage GC.

Nevertheless treatment paucity is due to chemoresistance which tighten feasible approaches.

Gold nanoparticles (AuNPs) have emerged as unique photothermal transducers, due to their innate bio-inertness and peculiar optical properties, and have attracted remarkable interest for cancer diagnostics and treatment such as optical HyperThermia. Moreover, when administered intravenously, nanoparticles accumulate preferentially in tumors than in normal tissue, since the pore size of the leaky and immature tumor blood vessels and the inefficient drainage system of tumors facilitate the passive extravasation of nanoparticles.

METHODS

Using the AGS gastric adenocarcinoma cell line (AGS WT) and its chemoresistant subpopulation obtained in our laboratory by chronic exposure to 5-fluorouracil (5FU), cisplatin (CISr), paclitaxel (TAXr) and to the FLOT regimen (FLOT_r), we evaluated response to AuNPs administration under two different conditions: normal pH and a condition that mimics extracellular tumor acidification environment. We also evaluated Carbonic Anhydrase IX (CAIX) levels in WT and resistant cell lines.

RESULTS

GC cells were able to efficiently uptake AuNPs under normal and acidic pH conditions with a surprisingly enhanced AuNP internalization under the latter, as testified by optic microscopy images and ICP quantification. Moreover, CAIX resulted upregulated in resistant cells with respect to WT.

CONCLUSIONS

CAIX is a tumor-associated transmembrane metalloenzyme crucial to maintain a favorable intracellular pH for tumor cell survival, growth and metastasis transferring acidity from the intra to the extracellular tumor microenvironment. Since CAIX is upregulated in chemoresistant and metastasizing GC tumor, our discovery that AuNPs can efficiently be uptaken by GC cells in both normal and acidic environments suggests that AuNPs have the potential to be effective therapeutic agents for GC, even in advanced or refractory stages.

CIRCULATING CD137+ T CELLS AS AN IMMUNE BIOMARKER FOR RESPONSE TO ANTI-PD1 IMMUNOTHERAPY IN NSCLC PATIENTS

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BACKGROUND-AIM

Anti-PD1 treatment, administered alone (PDL1>50%) or in combination with chemotherapy (PDL1<50%), is the standard of care in the first-line setting in non-oncogenic addicted Non-Small Cell Lung Cancer (NSCLC). Despite the success of these treatments, only 20-30% of these patients fully respond to these therapies, highlighting the need to identify novel biomarkers to optimize treatment strategies. CD137+ T cells are identified as activated and tumor-specific T cells. In several solid tumors, their levels correlate with responses to anti-PD1 therapy. In this study, we evaluate the levels of CD137+ T cells in NSCLC patients as biomarker of response to immunotherapy.

METHODS

PBMCs derived from 82 NSCLC patients undergoing both first- (from 2020-today) and second-line (from 2016 to 2019) anti-PD1 treatment were analyzed at baseline by cytofluorimetry for the expression of CD3, CD4, CD8, CD137, and PD1 molecules. These parameters were correlated with clinical outcomes.

RESULTS

Results show that in all patients, the levels of circulating CD137+T cells are significantly higher in responder patients (Rs) than in non-responders (NRs) ($p=0.02$), and that these high levels were ascribed to both CD8 ($p=0.02$) and CD4 ($p=0.03$) T cell populations. Moreover, patients with a percentage of CD3+CD137+>1.26% had prolonged OS ($p=0.007$) and PFS ($p=0.002$). Rs also showed high levels of CD137+PD1+T cells compared with NR ($p=0.002$) ascribed to both CD8 and CD4 T cells ($p=0.02$ and $p=0.03$, respectively).

Patients undergoing first- and second-line treatment were further analyzed and compared. The group of patients receiving first-line therapy had higher levels of CD137+ ($p=0.005$) and CD137+PD1+ ($p<0.001$) T cells compared with the other group, suggesting that naive patients had a more activated immune system.

CONCLUSIONS

Our results indicate that levels of circulating CD137+ T cells could predict the response to anti-PD1 therapy in NSCLC patients and could be used as biomarker for the success of anticancer treatment.

CIRCULATING CELL-FREE DNA (CFDNA) IN MEDULLARY THYROID CARCINOMA PATIENTS IS CHARACTERIZED BY SPECIFIC METHYLATION CHANGES AND FRAGMENTATION SIGNATURE

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BACKGROUND-AIM

Medullary Thyroid Carcinoma (MTC) is a rare neuroendocrine tumor, characterized by a poor prognosis in patients with regional and distant spreading. The diagnosis includes evaluation of calcitonin (Ct) in the biopsy washout and in the patients' serum, and the detection of residual disease during follow-up is performed with serum levels of Ct. These procedures however present limitations linked to wide fluctuation in Ct levels and the possible misdiagnosis of other diseases characterized by high levels of Ct. In this context, we investigated circulating DNA epigenetic features as potential additional biomarkers, specifically through fragmentation signature analysis and methylation changes analysis.

METHODS

Droplet digital PCR (ddPCR) was performed on cfDNA isolated from the plasma of MTC patients at different stages of the disease and healthy subjects.

Regarding the circulating DNA fragmentation analysis, we investigated three sizes of fragments of the human olfactory receptor genes and calculated the short fragment fraction (SFF) ratio.

For methylation analyses, cfDNA was subjected to bisulfite conversion before ddPCR.

Statistical analyses were performed to identify significant differences among groups and ROC curves were calculated.

RESULTS

In silico analyses of publicly available methylation arrays of MTC tissues and normal thyroid led us to select and further evaluate a hypermethylated CG dinucleotide, CG_16698623, in MTC.

SFF ratio and the methylation of CG_16698623 resulted in a significantly increased level in the pre-surgery plasma of MTC patients, with higher levels in tumors with extrathyroid extension, and ROC curve analyses resulted in an Area Under the Curve of 0.87 for SFF and of 0.89 for CG_16698623.

Patients at follow-up with either clinical remission, stable biochemical or structural disease showed no significant difference in the SFF ratio to healthy subjects.

CONCLUSIONS

Despite the small number of patients analyzed, due to the rarity of the disease, our data strongly suggest a diagnostic and prognostic value of cfDNA features, that could possibly guide clinicians in better MTC patients' management.

THE DNA DAMAGE RESPONSE TO CLASTOGENIC DRUGS DOES NOT AFFECT PRIMARY CILIOGENESIS OR NBS1 LOCALIZATION AT THE CENTROSOME/BB

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BACKGROUND-AIM

DNA and centrosome integrity is fundamental to maintain genome stability, avoid cell death and prevent tumor transformation. Therefore, in presence of DNA or centrosome damage, cells activate the DNA damage response (DDR) and the centrosome damage response, respectively, to block cell cycle progression. Since DNA damage may induce centrosome alteration (e.g. hyperduplication), increasing literature supports the possibility that the DDR controls cell cycle progression also by regulating the activation or localization of centrosomal proteins.

NBS1 is a member of the MRE11/RAD50/NBS1 (MRN) complex, that plays a crucial role in the DDR. Mutations in NBS1 lead to the DDR-defective Nijmegen Breakage Syndrome (NBS) characterized by microcephaly, immunodeficiency and cancer predisposition.

Importantly, NBS1 localizes at the centrosomes, prevents their hyper-duplication and regulates centriole separation. We recently discovered that NBS1 also localizes at the Basal Body (BB) and that its depletion lengthens Primary Cilia (PC) and affects their morphology and functionality.

Since MRN proteins are known to form a nuclear complex essential for their function in the DDR, we asked whether NBS1 localization and function at the centrosome/BB may require MRN complex assembly and/or may be influenced by DNA damage. Moreover, we evaluated whether the DNA damage influences primary ciliogenesis.

METHODS

We used cycling or starved human RPE-1 cells to analyze NBS1 centrosome/BB localization following MRE11 depletion via RNAi or pharmacological inhibition with mirin, and after the exposure to clastogenic drugs. In the same contexts, we further evaluated the frequency of ciliated cells and PC length and morphology.

RESULTS

We report that NBS1 localizes at the centrosome/BB independently on the MRN complex since neither MRE11 knock-down nor its inhibition prevents its centrosomal localization. Moreover, clastogenic drugs do not reduce NBS1 localization at the centrosome/BB nor affect PC frequency, length and morphology.

CONCLUSIONS

Our data indicate that NBS1 localizes at the centrosome/BB independently on both MRN complex assembly and DDR. Moreover, they support the existence of a new uncanonical role of NBS1 in the regulation of PC, which is not a mere consequence of DDR activation.

COX-2 INHIBITION COUNTERACTED TMZ-INDUCED MACROPHAGE RECRUITMENT IN GLIOBLASTOMA SPHERES BY OSTEOPONTIN DOWN-REGULATION.

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BACKGROUND-AIM

Recently, knowledge of the tumor microenvironment (TME) role in glioblastoma (GBM) is being highly increasing. GBM is a devastating disease characterized by a unique microenvironment that contributes to the tumor resistance. We previously pointed out the COX-2 overexpression induced by the chemotherapy drug Temozolomide (TMZ) in T98G (TMZ-resistant cell line) supported chemoresistance. Of note, COX-2 inhibition in a drug combination approach counteracted the TMZ effect. The GBM TME is enriched of neoplastic and non-neoplastic cells, such as newly recruited macrophages which ably establish the immunosuppressive TME secreting cytokines, chemokines, and growth factors. The extracellular matrix protein osteopontin (OPN) is crucially involved in TME regulating and in other processes like the macrophage's recruitment and polarization, stemness phenotype, and chemoresistance. The aim of this study was to elucidate whether the COX-2 inhibition by selective COX-2 inhibitor (Celecoxib - CXB) could affect the TME modulating the OPN levels and its receptor, CD44, a well-known stemness marker in GBM.

METHODS

Human GBM cell line T98G and a primary culture, derived from a resected GBM tissue, both TMZ-resistant cells, were exposed to CXB, TMZ, and their combination for 72 hours and then cocultured with macrophage cell line U937 in tumorspheres' medium until their generation. Levels of OPN and CD44 were evaluated by Elisa kit and Western blot. To better define COX-2 involvement in TMZ resistance exogenous prostaglandin 2 (PGE2), the COX-2 downstream mediator, was also added to cultures.

RESULTS

In macrophages-infiltrated tumorspheres, TMZ enhanced the OPN and CD44 expression. Of note, CXB+TMZ significantly counteracted the TMZ effect lowering both the OPN and CD44 levels. Surprisingly, when exogenous PGE2 was added to the drug combination, the OPN and CD44 levels increased in the GBM cell line and primary culture, highlighting the COX-2 role in immunosuppressive TME promotion and in the macrophages' recruitment.

CONCLUSIONS

Overall, our findings in GBM resistant cells strongly suggest that pharmacological inhibition of COX-2 combined with TMZ counteracts the TMZ-induced OPN upregulation. Our findings emphasize the crucial role of the COX-2-PGE2-OPN axis as an attractive and potent therapeutic target for GBM treatment.

ROLE OF DANGER AND MICROBIAL SIGNALS IN NEUTROPHIL SUBPOPULATIONS RECRUITMENT DURING INFECTION.

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BACKGROUND-AIM

Neutrophils are key cells in the innate immune response, but the exact molecular mechanism of their recruitment during infection is unclear. While their migration to infected sites is traditionally associated with pathogen recognition via Pattern Recognition Receptors (PRRs), recent discoveries have identified new neutrophil subpopulations in the vasculature at steady state. Mechano-sensing has also emerged as a factor in the response to gram-negative bacteria, independent of PRRs.

Our project aims at elucidating neutrophil recruitment during infection and at investigating subpopulations at the infected site.

METHODS

We used a skin infection model with *Candida albicans* fungus, *Staphylococcus aureus* gram-positive bacteria, and *Pseudomonas aeruginosa* gram-negative bacteria in wild-type mice and mice deficient in interleukin receptors and PRR signaling. Flow cytometry identified neutrophils as Ly6G^{high/+} Ly6C^{int} CD11b⁺ cells, with fresh subpopulations expressing CD62L^{+ /high} CXCR4^{low} and aged subpopulations lacking CD62L but expressing CXCR4. To understand the molecular cascade underlying neutrophil recruitment, we quantified pro-inflammatory mediators using qPCR, ELISA, and flow cytometry.

RESULTS

Findings revealed that neutrophil recruitment is kinetically regulated by upstream mediators converging to MyD88. Two waves of neutrophil recruitment were observed: an early wave within 6-8 hours mediated by an LTB4-IL-1 axis, independent of infection type. Neutrophils were recruited via an IL-1-CXCL1 axis, and CD11c⁺ immune cell depletion affected only early recruitment. PRRs had minimal contribution to this wave, while preliminary data suggest a role for mechanosensors, particularly PIEZO 1, in regulating early neutrophil recruitment. Regarding neutrophil subpopulations, both fresh and aged neutrophils were identified at the infected site during the early wave, but a shift towards aged and activated (CD62L⁻ CXCR4⁻) neutrophils was observed later in recruitment.

CONCLUSIONS

We propose a model of early neutrophil recruitment independent of pathogens, relying on a lipid-cytokine-chemokine axis with minimal PRR contribution. This mechanism, already associated with neutrophil recruitment during chronic sterile inflammation, seems to be employed during infections as well.

COMBINATION OF CRISPR/CAS9 SYSTEM AND ORGAN-ON-A-CHIP TECHNOLOGY RECAPITULATED CYSTIC FIBROSIS AIRWAY INFLAMMATION IN A BRONCHIAL EPITHELIAL CELL LINE AND REVEALED CFTR-RELATED EPITHELIAL CELL MIGRATION.

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BACKGROUND-AIM

Cystic Fibrosis (CF) is a genetic disease caused by mutations in the CFTR gene, leading to chronic and unresolved inflammation in the airways. Despite the availability of immortalized airway cells, most cellular models in CF are engineered to overexpress the wild type (WT) form or specific mutations of the CFTR gene. However, such models may show altered CFTR-related pathways and networks. To examine CFTR-controlled pathways, we generated CFTR knockout (KO) clones of the bronchial cell line 16HBE14o-, using the CRISPR/CAS9 technology.

METHODS

CRISPR/CAS9 ablation of CFTR was obtained by targeting the exon 1 of the CFTR gene. DNA-sequencing and western blotting confirmed mutations and the absence of protein. Proteomics was carried out in n=3 WT and n=3 KO biological replicates by nanoLC-Orbitrap-MS/MS analysis. Differentially expressed proteins were analyzed by hierarchical clustering analysis. Supernatants of cells treated or not with poly(I:C) were processed by Luminex assay for cytokine quantification. Neutrophil and epithelial cell migration studies were carried out in microfluidic S1 chips (Emulate®) populated by lung endothelial cells in the bottom channel and KO or WT 16HBE14o- clones in the top channel. Migration was evaluated 3 days after co-culture by confocal imaging.

RESULTS

KO clones showed no CFTR expression and activity. Proteomic analysis revealed 1969 common proteins between WT and KO clones and alteration of pathways related to cell adhesion, viral infection, immune cell recruitment, cell death and cancer in KO clones. These cells also showed 74 up-regulated and 88 down-regulated proteins with a p value ≤ 0.05 and released higher amounts of the pro-inflammatory cytokines IL1 α , IL12p40, IL7, TNF α , VEGFA, RANTES, IL3, IP10, IL6 and IL8. Higher levels of eotaxin, IL1 α , IL1 β , IL12p40, TNF α , VEGFA, IP10, IL3, IL7 and IL8 were found in KO cells after exposure to poly(I:C). Moreover, KO cells showed higher capability to recruit neutrophils (p < 0.01) and to invade the bottom channel of the airway chip.

CONCLUSIONS

In conclusion, KO cells recapitulated CF inflammation and showed epithelial cellular movement, thus emphasizing the effect of a dysfunctional CFTR on cell junctions and suggesting new insights for investigating more deeply CFTR involvement in lung cell migration.

INVOLVEMENT OF COX-2 IN ESTABLISHING AN IMMUNOSUPPRESSIVE MICROENVIRONMENT IN TUMORSPHERES DERIVED FROM TMZ-RESISTANT GLIOBLASTOMA CELLS.

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BACKGROUND-AIM

Cyclooxygenase 2 (COX-2) is a critical component in fuelling glioblastoma (GBM) proliferation, maintenance of stemness features, and chemoresistance. Our group recently demonstrated that the COX-2 upregulation induced by Temozolomide (TMZ) treatment in T98G (TMZ-resistant cell line) supported chemoresistance, and COX-2 transfer in the recipient macrophages by T98G-released extracellular vesicles, promoting an M2 polarization. Of note, COX-2 inhibition counteracted these effects. GBM is characterized by an immunosuppressive tumor microenvironment (TME), strictly associated with therapy resistance, and in which macrophages represent the most abundant cells sustaining tumor progression. The aim of this work was to explore the ability of TMZ-induced COX-2 to influence the immunosuppressive TME in the GBM context using an in vitro model of heterotypic cell interaction between tumorspheres derived from GBM cells and human macrophages U937.

METHODS

TMZ-resistant T98G cells and GBM primary cultures were exposed to Celecoxib (CXB-selective COX-2 inhibitor) in combination or not with TMZ and then grown in tumorspheres' complete medium in the presence of U937 cells until spheres generation. The tumorsphere formation and the macrophage infiltration levels within tumorspheres were evaluated by optical and confocal microscopy. The macrophage polarization was evaluated by quantifying TGF β -1 and IL-1 β levels through ELISA kit and CD206 expression by flow cytometry.

RESULTS

TMZ treatment strongly increased tumorsphere size and macrophage infiltration. Of note, CXB+TMZ exposure markedly reduced the tumorsphere growth and the macrophage infiltration compared to TMZ alone. Moreover, TMZ induced an M2 polarization evaluated by TGF β -1 and CD206 levels, effect significantly counteracted by CXB, that reduced M2 status and increased IL-1 β , an M1-related marker.

CONCLUSIONS

In this work, we report evidence of the ability of TMZ to induce the increase in tumorsphere size and the promotion of the immunosuppressive microenvironment in the context of resistant GBM cells. These effects seem to be mediated by TMZ ability to upregulate COX-2 since the inhibition of this enzyme contrasted both TMZ-induced effects.

FUNCTIONALIZED MAGNETIC NANOPARTICLES FOR THE THERAPY OF ADIPOSOPATHY BY MAGNETO-MECHANICAL EFFECT

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BACKGROUND-AIM

Adiposopathy or "sick fat" is defined as adipose tissue dysfunction promoted and/or worsened by visceral fat accumulation and sedentary lifestyle. It is characterized by adipocyte hypertrophy and can be considered the primary cause of most cases of adiposity-related metabolic diseases such as Type 2 diabetes mellitus, hypertension, dyslipidemia and all major cardiovascular diseases (CVD). Current treatment involves lifestyle modification, pharmacotherapy, surgery, and treatment of obesity-related complications.

In this work we investigate a new treatment of adiposopathy using magnetic nanoparticles (MNPs) in combination with magneto-mechanical stress by an external alternating magnetic field (AMF). The exposure to AMF leads to mechanical oscillations of the MNPs and consequently to cellular damage. This approach has been tested on the two cell types mainly involved in adiposopathy, namely macrophages and adipocytes.

METHODS

The Fe₃O₄ MNPs were tested alone or in association with AMF on mature adipocytes obtained from differentiated ADSC cells and on RAW 264.7 macrophages.

Intracellular presence of neutral lipids was highlighted with red oil in adipocytes after treatment, and cellular viability were monitored with time lapse and with trypan blue exclusion test.

MNPs intracellular uptake was evaluated by TEM analysis and measured by a Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). Then cellular viability, ROS and levels of DNA stand breaks were detected.

RESULTS

The exposure of RAW264.7 to MNPs + AMF treatment has been associated to a reduced cell viability and to increased levels of ROS and cellular damage.

Mature adipocytes obtained from ADSC cells showed signs of cellular damage after MNPs + AMF as demonstrated by the presence of cellular vacuoles. Furthermore, the treatment induced a marked fragmentation of the lipid drops due to lipolysis process.

CONCLUSIONS

These results showed that the MNPs internalization is a crucial process to cause cellular damage and that the magnetic field exposure enhanced this effect in both macrophages and adipocytes, even if macrophages showed greater resistance than adipocytes. Therefore, it is possible to state that MNPs + AMF determines an inhibition of the differentiation of mesenchymal cells into adipocytes and in parallel the activation of the processes of lipolysis, which could lead to the restoration of the cellular functions of the adipose tissue.

UNRAVELING CELLULAR SENESENCE OF EPICARDIAL ADIPOSE TISSUE IN TYPE 2 DIABETES

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BACKGROUND-AIM

With the progressive increase of Type 2 Diabetes (T2D), its cardiovascular complications are rising. Patients with T2D have a risk of death from cardiovascular causes that is two to six times that among persons without T2D. Epicardial adipose tissue (EAT), the visceral fat of the heart, may play an active role in dysregulation of cardiac function. EAT thickness positively correlates with the release of inflammatory molecules and with the severity of heart pathologies, including heart failure, aortic valve stenosis and coronary artery disease (CAD). In patients with diabetes, prolonged hyperglycemia damages several organs, including heart. Several evidences suggest that EAT can act as a transducer to mediate the effects of systemic inflammation to the myocardium. Thus, potent pro-inflammatory activation of EAT suggests a direct involvement of cardiac visceral fat in inflammatory phenomena occurring in patients with cardiovascular diseases.

This study aims at investigating whether different glucose concentrations may impact on EAT functions.

METHODS

Blood samples and EAT biopsies were collected from non-diabetic (ND) CAD patients (n=37) and T2D CAD patients (n=20) enrolled during coronary artery bypass surgery. Cytokines and chemokines secretion was measured using the Bioplex multiplex assay.

From a subgroup of patients, EAT-derived mesenchymal stem cells (MSCs) were isolated and cultured in high glucose (HG 25 mmol/l) or normal glucose (NG 5.5 mmol/l) concentration. mRNA levels were measured by real time RT-PCR.

RESULTS

IL-1beta, IFN-gamma, IP10 and Eotaxin were increased in serum from T2D CAD patients. In parallel, EAT biopsies from diabetic CAD patients secrete higher levels of pro-inflammatory cytokines, such as IL-6, IL-8, IL-1beta, TNF-alpha, MCP-1, compared to ND.

In EAT-derived MSCs, mRNA levels of the senescence markers p21CIP1/WAF1 and p16INK4A as well as the fibrosis-associated gene ACTA2 were significantly increased in HG, compared to NG. Consistent with these data, a senescence-associated secretory phenotype (SASP) was observed in HG-treated MSCs.

CONCLUSIONS

Collectively, these data indicate that a diabetic-like environment directly affects the hallmarks of senescence and fibrosis of EAT-derived MSCs.

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NON-GENOMIC ANDROGEN ACTION IN TRIPLE NEGATIVE BREAST CANCER

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BACKGROUND-AIM

Triple negative breast cancer (TNBC) represents the 15-20% of all breast cancer subtypes and is characterized by the lack of estrogen receptor and progesterone receptor as well as the absence of type-2 epidermal growth factor receptor overexpression. Chemotherapy remains the mainstream treatment, because of the paucity of alternative targets. Many studies have tried to identify the TNBC biomarkers. Among them, the androgen receptor (AR) is gaining a prominent position. The receptor is, indeed, expressed in 20–50% of TNBCs and regulates several pathways affecting the disease's aggressiveness

METHODS

In this study, we have used TNBC-derived MDA-MB231 and MDA-MB453 cells that express AR. BrdU incorporation, wound healing and Trans-well assays show that androgen challenge induces invasion and proliferation of these cells. The antiandrogen, Bicalutamide or two small peptides, S1 and Rh2025u designed to mimic the AR sequences responsible for interaction of the receptor with Src or Filamin A, respectively, reverse the androgen-induced effects in both cell lines. Biochemical approaches show that the ligand-bound AR interacts with Src or Filamin A thus controlling cell invasion and proliferation, respectively. Treatment of MDA231-BALBc xenografts with Rh2025u significantly reduces the tumor growth.

RESULTS

Our findings demonstrate that AR specifically interacts with different partners in androgen-treated TNBC cells. Complexation of AR with Src triggers invasion, while the AR/FlnA complex regulates the TNBC cell growth (1 and in press)

CONCLUSIONS

The present study highlights the pivotal role of AR in TNBC progression and supports the use of the S1 and Rh2025u peptides in TNBC treatment.

MGL LECTIN RECEPTOR AND IMMUNOSUPPRESSION NETWORKS IN GLIOBLASTOMA

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BACKGROUND-AIM

Aberrant glycosylation sustains tumor progression and immunogenicity¹. Alterations in the mucin O-glycosylation pathway generate truncated Tn and its sialylated form STn antigens. Tn is recognized by the C-type Macrophage Galactose-Type Lectin (MGL) expressed on dendritic cells and macrophages (MDM). MGL-Tn interactions negatively modulate the antitumor immune response and promote tumor evasion, thus their targeting has been proposed as immunotherapeutic approach in cancer¹. Glioblastoma (GB) is the most aggressive and still untreatable brain tumor. Hypoxia, impaired angiogenesis and immunosuppressive cells (myeloid-derived suppressor cells (MDSC), microglia (MG), MDM) contribute to mediate the immunotherapy resistance¹. In an engineered Tn-expressing GB mouse model, the ortholog *mg1* has been detected on CD163+ PD-L1+ MDM2, while no evidence is available in human GB². Dissecting the immunosuppressive mechanisms in GB is crucial to identify novel therapeutic targets.

METHODS

Patient derived tumor samples (15 GB;3 low-grade glioma (LGG)) were collected at the Neurosurgery Dpt, Umberto I Hospital in Rome, and freshly processed as single cell suspension to characterize MGL expression on the different immune cell subsets (MDSC, MDM, MG) by flow-cytometry. Tn, STn and CD163 expression was analyzed on formalin-fixed paraffin embedded (FFPE) tissue sections (26 GB, 8 LGG) by IHC.

RESULTS

Our preliminary results show that MGL is heterogeneously expressed on myeloid immune cells and its overall expression is higher in GB vs LGG. Interestingly, MGL is expressed not only by MDM, but also by MDSCs and MG. In accordance, Tn antigen displayed a similar expression profile by being only captured in GB and in association to neoplastic cells, while it was completely absent in LGG. Tn expression positively correlates to CD163 expression in GB. STn was expressed in both GB and LGG microenvironment in correspondence of vasculature compartment.

CONCLUSIONS

Our results suggest that, depending on the spatial and cellular distribution, MGL-Tn axis may be relevant in immunosuppression in GB. Further studies are required to evaluate the impact of MGL engagement by the two ligands on the immunosuppressive cells. The MGL-Tn axis may represent a novel therapeutic strategy for GB.

COMBINATION OF CHEMOTHERAPY AND POSTBIOTICS: AN EXPERIMENTAL INTEGRATED APPROACH TO IMPROVE RESPONSE TO TREATMENT IN COLORECTAL PATIENT-DERIVED TUMOR ORGANOIDS

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BACKGROUND-AIM

Several studies have demonstrated the beneficial effects of probiotics and gut microbiota modulation in patients treated with anticancer pharmacological treatments. Besides the well-established use of probiotics in managing chemotherapy-related gastrointestinal disorders, in a previous study, we demonstrated the potential direct antiproliferative effects of the cell-free supernatant of *Lactobacillus rhamnosus* GG (LGG-CFS) on colorectal cancer cells as well as its synergistic effects when concurrently administered with standard CRC chemotherapy. To further evaluate the mechanisms by which LGG may counteract CRC progression, the effects of LGG-CFS were here tested on both drug-sensitive and resistant CRC patient-derived tumor organoids (PDTOs).

METHODS

Drug-sensitive CRC PDTOs were treated with increasing doses of 5-fluorouracil (5-FU), oxaliplatin (OX) and irinotecan (IRN) in combination with a fixed dose of LGG-CFS. Cell proliferation was evaluated by using the CellTiter-Glo assay. Western blot (WB) experiments were performed on treated organoids to evaluate the effects of LGG-CFS on cancer-related signal transduction and apoptotic pathways. Similarly, drug-resistant CRC PDTOs were treated with both chemotherapy and LGG-CFS to evaluate if LGG can revert or reduce drug resistance.

RESULTS

The results previously obtained in 2D CRC cell lines showed a cytostatic effect induced by LGG-CFS as observed by flow cytometry. These results were here confirmed by treating CRC PDTOs with LGG-CFS alone and in combination with chemotherapies demonstrating the antiproliferative potential of LGG-CFS which lowered the PDO diameter. In addition, a synergistic effect of LGG-CFS was also observed when concomitantly administered with 5-FU, OX and IRN. WB analyses confirmed the cytostatic effects of LGG as no activation of apoptosis was observed by measuring Caspase-8 and PARP markers. Finally, LGG-CFS partially reverted the drug resistance when concomitantly administered with chemotherapy.

CONCLUSIONS

LGG-CFS showed antiproliferative and synergistic effects also in advanced CRC PDTOs models suggesting how LGG could be proposed as an adjuvant for the development of integrated treatment approaches to reduce chemotherapy side effects and overcome drug resistance in CRC.

THE CG17199325 METHYLATION HOTSPOT OF THE SLC22A17 GENE AS POTENTIAL EPIGENETIC BIOMARKER IN CUTANEOUS MELANOMA

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BACKGROUND-AIM

Several studies have highlighted the role of tumor microenvironment (TME) in cancer hallmarks, including extracellular matrix degradation, tumor invasion, and metastasis, mediated by NGAL-MMP9 (Neutrophil Gelatinase-Associated Lipocalin – Matrix Metalloproteinase 9) complex, as well as iron metabolism, regulated by NGAL-SLC22A17 (Solute Carrier Family 22 Member 17) complex. In this context, DNA methylation (methDNA) may affect the expression of TME-associated genes leading to cancer progression. On these bases, this study aimed to investigate the role of promoter and intragenic methDNA in the regulation of SLC22A17 expression in cutaneous melanoma (CM).

METHODS

The assessment of the SLC22A17 expression levels and methDNA profile in CM was performed by using TCGA and GTEx datasets. The correlation between gene expression and methDNA was also evaluated in vitro on 7 melanoma cell lines. Moreover, the identified SLC22A17 methDNA hotspot was analyzed in FFPE melanoma and nevi samples by the custom Methylation-Sensitive Restriction Enzyme-droplet digital PCR (MSRE-ddPCR) assay.

RESULTS

The bioinformatic analyses revealed that SLC22A17 was significantly downregulated in CM compared to nevi. Interestingly, the SLC22A17 intragenic methDNA was positively correlated with its gene expression. The regulatory role of methDNA on SLC22A17 expression was also confirmed in melanoma cells treated with the demethylating agent 5-azacytidine. Moreover, the MSRE-ddPCR analysis demonstrated that the methDNA levels of the down-stream promoter cg17199325 hotspot were significantly higher in melanoma samples compared to controls. Of note, cg17199325 methDNA levels were also positively correlated with different clinicopathological characteristics, including stage, Breslow depth, number of mitosis, vascular invasion, and ulceration.

CONCLUSIONS

Overall, the obtained results showed that SLC22A17 could act as tumor suppressor gene, highlighting the regulatory role of methDNA on its gene expression. Moreover, the cg17199325 hotspot could represent a promising epigenetic biomarker for the diagnosis and prognosis of CM.

THE CHARACTERIZATION OF THE UNKNOWN GENETIC BASES OF BRUGADA SYNDROME EXPLOITING WHOLE EXOME SEQUENCING

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BACKGROUND-AIM

Brugada Syndrome (BS) is an inherited arrhythmogenic disease with a 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% of patients remain genetically undiagnosed. To identify new candidate genes, we performed Whole Exome Sequencing (WES) of 200 patients, both sporadic and familiar cases.

METHODS

WES was performed on the NovaSeq Illumina Platform (mean coverage: 180X, 97% target region >20X). Reads were analyzed exploiting the Dragen BioIT Platform, coding and splice regions variants ($MAF \leq 0.01\%$) prioritized and classified according to ACMG guidelines with the support of eVai-EnGenome software. In addition, the burden test with Fisher exact test allowed us to extrapolate genes with higher mutation burden, suggesting a possible pathogenic role. A clinical database for the enrolled patients contains all the clinical information with a complete follow-up for more than 10 years.

RESULTS

WES analysis showed rare prioritized variants located in about 400 genes. Subsequent burden tests allowed us to focus on a smaller number of genes, confirming the role of SCN5A and highlighting possible associations 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, 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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BLOCKING THE CN-NFAT PATHWAY IN DENDRITIC CELLS ALTERS DIFFERENTIATION AND INDUCES TOLEROGENTIC PHENOTYPE

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BACKGROUND-AIM

The Nuclear Factor of Activated T Cells (NFAT) is a family of transcription factors activated by the calcium-calmodulin-dependent phosphatase Calcineurin (CN). Originally discovered in T lymphocytes, NFATs play critical roles in T cell activation and proliferation. However, our research revealed that NFATs are also activated in dendritic cells (DCs) through CD14 signaling upon lipopolysaccharide stimulation, leading to differentiation and apoptosis.

Considering this premise and emerging evidence highlighting the immunosuppressive role played by certain cell types like Myeloid-Derived Suppressor Cells in pathological contexts, our project aims to determine the involvement of the CN-NFAT pathway in early DC differentiation and its potential contribution to the development of an immunosuppressive phenotype.

METHODS

To explore this, we transfected a growth factor dependent splenic dendritic cell line called D1 with CN inhibitor peptide VIVIT using a lentiviral vector, generating DC-iCN. To determine the effect of the inhibition of the CN-NFAT pathway in DC-iCN we realized a proteomic analysis, and functional and metabolic assays. The potential immunosuppressive activity of the DC-iCN has been determined by Mixed Leukocyte Reaction assay. Lastly, we analyzed the potential effects of inhibiting NFAT in a mouse model through the intravenous administration of nanoparticles carrying the VIVIT peptide in order to investigate whether this inhibition would alter the hematopoietic process that leads to the formation of DCs

RESULTS

Blocking the CN-NFAT pathway in DCs increased their growth rate and altered the cell cycle, prolonging the G2/M phase and shortening the G1 phase. Metabolically, NFAT inhibition induced a marked Warburg effect, characteristic of rapidly proliferating cells. Moreover DC-iCN demonstrated a highly immunosuppressive phenotype, differentiating naive T cells into FOXP3+ regulatory T cells in MLR assays. Interestingly, inhibiting the CN-NFAT pathway in vivo expanded the granulocyte-macrophage progenitor involved in DC ontogeny.

CONCLUSIONS

These results suggest that blocking the CN-NFAT pathway alters early DC differentiation, acquiring characteristics of undifferentiated cells and a highly tolerogenic phenotype. Further analyses in humans will supplement these findings.

INNOVATIVE 3D MODELS TO CHARACTERISE THE HERG1/ β 1 COMPLEX AND THE STROMA'S ROLE IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

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BACKGROUND-AIM

Dysfunction of ion channels contributes to several diseases, including cancer, by mediating interactions between tumour cells and the tumour microenvironment (TME). The hERG1 channel, which is aberrantly expressed in cancer cells is coexpressed and interacts with β 1 integrins. To fully elucidate the complex and the role of the stroma in PDAC, we thoroughly investigated its expression and the interactions of CAFs and pancreatic stellate cells (PSCs) with PDAC cells, both in 2D and 3D, using multiple and innovative approaches.

METHODS

To assess the expression of the hERG1/ β 1 complex in all cell lines we performed co-immunoprecipitation and immunofluorescence experiments. For the migration experiments, PANC-1 cells plated on a desmoplastic matrix and time-lapse videos were analyzed by Amira® (ThermoFisher). For force measurements, hexagonal arrays micropillars were produced and coated with ECM proteins. The position of the pillar tops was observed by confocal microscopy and determined using custom software (Matlab, Mathworks). Forces were obtained by multiplying the pillar deflections by the array's characteristic spring constant. We applied to different 3D models: heterospheroids in ultra-low attachment plates and co-cultures with 3DProSeed hydrogel plates (Ectica Technologies) with PSC and patient-derived pancreatic CAFs, respectively.

RESULTS

Migration experiments of PANC-1 cells showed a significant reduction in cell area, translocation and migration after treatment with scDb, a bispecific antibody targeting the aforementioned complex. From the force measurements experiments, it was found that disruption of the complex interaction increased the average mean and maximal inward forces exerted by cells on a single pillar, particularly on 29 kPa substrates. These two 3D models showed us that pancreatic cancer cells undergo a morphological and behavioural change together with an alteration in the expression of pro-tumourigenic markers, such as CD105.

CONCLUSIONS

Our findings suggest that the interaction between the hERG1/ β 1 complex and ECM proteins affects the migration, force transduction and stiffness sensing machinery in PDAC cells. The association between tumour cell lines and PSC/CAF represents a good model to study morphological and behavioural changes in addition to tumourigenic markers expression in PDAC.

INVOLVEMENT OF THE RNA-BINDING PROTEIN HUR IN THE ACQUISITION OF RESISTANCE TO EGFR-TYROSINE KINASE INHIBITORS IN NON-SMALL CELL LUNG CANCER

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BACKGROUND-AIM

Advanced non-small cell lung cancer (NSCLC) patients with sensitive mutations in epidermal growth factor receptor (EGFR) are treated with tyrosine kinase inhibitors (TKIs), such as gefitinib and osimertinib. However, acquired resistance invariably develops, associated with immune suppressive phenotype involving expression of the cytokines IL-6 and IL-8. The RNA-binding protein HuR is an emergent regulator of cancer, currently studied for therapeutic targeting. Indeed, HuR enhances mRNA stability/translation of key effector genes, including cytokines, cell cycle regulators and anti-apoptotic factors. Here, we investigated the role of HuR in supporting NSCLC cell proliferation and survival in response to TKI treatment.

METHODS

EGFR-TKI-resistant cell lines (PC9GR/H1975OR) were generated by treating PC9/H1975 cells with gefitinib and osimertinib, respectively. Role of HuR was studied using PC9- and H1975-HuR-KO cell lines generated by CRISPR/Cas9 technology. Protein expression was analysed by immunoblotting, confocal microscopy and ELISA assay. Cell cycle and apoptosis were analysed by flow cytometry.

RESULTS

In silico analyses of public databases showed significant upregulation of both HuR mRNA and protein levels in lung adenocarcinoma primary tumors respect to normal tissue.

Gefitinib treatment reduced HuR expression in TKI-sensitive but not in resistant cells, in which HuR protein was more localized in the cytoplasm. Depletion of HuR impaired in vitro TKI-resistance acquisition to both gefitinib and osimertinib in PC9 and H1975 cells, respectively, together with a significant increase of the apoptotic rate induced by the TKIs. Cell proliferation was reduced in PC9- and H1975-HuR-KO cells respect to parental cell lines according to significant cell cycle accumulation in G0/G1 phase, increased levels of p21^{WAF1} protein and downregulation of Cdk2 and Cdk6. Additionally, loss of HuR protein reduced IL-6 and IL-8 secretion, as well as cell migratory ability, associated with the acquisition of TKI-resistance.

CONCLUSIONS

Our findings indicate that HuR (i) contributes to the acquisition of TKI-resistance limiting TKI-treatment-induced apoptosis and sustaining cancer cell migration; (ii) promotes the secretion of cancer-associated cytokines favoring an immunosuppressive tumor microenvironment in NSCLC.

JAGGED LIGANDS IN THE EXTRACELLULAR VESICLES-MEDIATED ANGIOGENESIS AND OSTEOCLASTOGENESIS IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Multiple myeloma (MM) is a still incurable hematological disease mainly due to the pathological interaction with the bone marrow (BM) niche. The aberrant expression of Notch pathway members, i.e. Notch2 and its ligands Jagged1 and 2, contributes to the proliferation and survival of MM cells, and to the pro-tumoral behaviour of BM cells leading to osteoclastogenesis and angiogenesis.

Extracellular vesicles (EV) shed by tumor cells are new key players in the communication between MM and its microenvironment. Previously, we reported of the functional effect of Notch2 transferred by MM cell-derived EV (MM-EV) in the BM niche, exploring osteoclastogenesis and angiogenesis. In this work we explore the role of the vesicular Jagged 1 and 2.

METHODS

To assess the role of Jagged 1 and 2 in EV-mediated communication, EV were isolated from the MM cell lines RPMI8226 and OPM2 knocked down or not for Jagged1 and 2 (MMSCR-EV MMJ1/2KD-EV). MM-EV and MMJ1/2KD-EV were characterized for their ligands expression as well as their ability to activate Notch signaling in recipient cells by two reporter assays performed on HeLa cells and on Notch-reporter Tg(T2KTp1bglob:hmgf1-mCherry)jh transgenic zebrafish embryos. The osteoclastogenic ability of MM-EV and MMJ1/2KD-EV was assessed on the RAW264.7 cell line and the angiogenic activity by a tube formation assay on human pulmonary artery endothelial cells.

RESULTS

Our results demonstrated that MM-EV carry Jagged1 and 2 ligands, which induce Notch signaling activation in recipient cells as well as osteoclastogenesis and angiogenesis on monocytes and endothelial cells, respectively.

CONCLUSIONS

MM-EV promote osteoclastogenesis and angiogenesis in a Jagged-dependent way. Thereby targeting Jagged-mediated Notch pathway activation may represent a promising strategy to hamper the pro-tumorigenic activity of MM-EV.

CHARACTERIZATION OF SARS-COV-2 ENVELOPE PROTEIN AS POTENTIAL MODULATOR OF CALCIUM HANDLING IN HUMAN IPSCS AND CARDIOMYOCYTES MODELS

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BACKGROUND-AIM

COVID-19 is now considered a multiorgan disease in which impaired respiratory function is often accompanied by cardiovascular disorders. In particular, an increased cardiac arrhythmogenesis associated with COVID-19 has been observed, probably associated to a direct invasion to the cardiac tissue by the virus. In support of this thesis, the presence of virus envelope (E) protein was observed on patients cardiomyocyte membranes, where it is supposed to act as ion channel.

This suggests that E protein may affect the electrical properties of the myocardium, and thus impair the normal cardiac electrophysiology.

METHODS

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

RESULTS

We have first generated lentiviral vectors for the overexpression of SARS CoV 2 E protein and validated the vector on HEK293 cells. In addition, we obtained human induced pluripotent stem cells (hiPS-Cs) stably expressing the construct. On these cellular models we have assessed the transgene expression by PCR and ICC assays, performed Ca²⁺ imaging experiments and characterized the proliferation rate. Results The expression of E protein was confirmed on both cell models, showing a clear localization in the cytoplasm as well on the plasma membrane. E protein overexpression resulted in a different cell Ca²⁺ handling in response to drug (Tapsigargin) able to empty intracellular stores. In line with this effect, cell proliferation rate was modified by E protein overexpression.

CONCLUSIONS

Our results demonstrate E protein localizes both in human intracellular and plasma membranes, where it acts as ion channel protein. In this function, E protein deranges intracellular Ca²⁺ handling, leading to a marked depletion of intracellular Ca²⁺ pools. Future experiments will assess whether the alteration on intracellular Ca²⁺ stores induces modifications of plasma membrane excitability, laying the basis to better explain cardiac arrhythmogenesis associated with Covid-19.

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UNI4ME PROJECT: THE EVALUATION OF LEVELS OF HEAVY METALS IN THE BLOOD OF A YOUNG POPULATION AND THE CORRELATION WITH LIFESTYLE HABITS.

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BACKGROUND-AIM

Uni4Me project is an epidemiological screening for 7 heavy metals (Pb, Cd, Cr, Co, Hg, Ni, Zn) in the blood of a healthy cohort. The goals are 1) to assess the baseline for each metal in not intoxicated and not exposed young people and 2) to perform a correlation between the detected levels of heavy metals and habits. The outcome is the identification of reference thresholds that could be unsafe in a healthy population, due to sub-chronic exposure, which can lead to toxicity in association with environmental risk factors.

METHODS

The protocol study was approved by the Ethical Committee. The 154 peripheral blood samples were collected at two different time points, when a structured digital interview was proposed to collect information about lifestyle and habits. Regarding the statistical analysis, the comparison of the distribution of the metal levels among groups were performed with the Mann-Whitney test, in the case of two groups, while with the Kruskal-Wallis test (followed by a post-hoc analysis with the Dunn's test) in case of more than two groups. The groups were defined based on the lifestyle and habits information. Furthermore, dietary patterns were identified from dietary data by applying a clustering method for ordinal data (Biernacki and Jacques, 2016) and then their metal levels were compared.

RESULTS

Among lifestyle information, we found that smoking traditional cigarettes was significantly associated with higher levels of both Pb ($p=0.0051$) and Cd ($p<0.0001$), while all kinds of smoking only with higher levels of Cd ($p<0.0001$). Moreover, wearing earrings or piercing and using hair dye were both associated with higher levels of Co ($p=0.0064$ and $p=0.0018$, respectively). Overweight was associated with higher levels of Pb ($p=0.0002$). Regarding drugs, the habitual usage of nonsteroidal anti-inflammatory drugs was also associated with higher levels of Cd ($p=0.0022$). A dietary pattern, characterized by a low consumption of meat and fish and a high consumption of vegetable, showed lower levels of Hg. Moreover, the consumption of milk was associated with lower levels of Cd ($p=0.0022$).

CONCLUSIONS

The project is a starting point for the innovation of the evaluation of metals exposure, as, the association with environmental exposure will lead to the identification of actionable factors to reduce the risk of toxic burden in young and healthy people and to improve knowledge of the potential role of metals in human disorders.

PLASMA METABOLOMICS OF NEURAL CREST CANCER PATIENTS AS A DIAGNOSTIC TOOL

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BACKGROUND-AIM

Paraganglioma (PGL) and glioblastoma (GBM) are highly vascular neural crest tumors composed of glial and neuronal cells. Affected individuals are identified in hospital outpatient settings via PET/CT (involving ionizing radiation) or MRI (involving strong magnetic fields) and must undergo life-long surveillance using these techniques. Recently, plasma metabolomics has proven useful for identifying metabolomic profiles associated to paraganglioma and glioblastoma. Using rapid, inexpensive targeted FIA-MS/MS of plasma or dry blood spots, as in newborn metabolic screening, we recently reported a metabolic signature putatively specific for head and neck paraganglioma (HNPGGL).

METHODS

We screened 57 plasma metabolites comprising amino acids, acyl-carnitines, lysophosphatidylcholines and succinylacetone, obtained from 52 GBM and 20 low grade gliomas (LGG) compared with 59 HNPGGLs and 24 healthy controls previously analyzed. The screening was performed by flow injection analysis using tandem mass spectrometry (FIA-MS/MS) on the RenataDXTM Screening System FIA platform. Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) were performed on SIMCA® 17 Multivariate Data Analysis Software (Sartorius). ROC analysis was performed on MetaboAnalyst 5.0.

RESULTS

Our targeted metabolomics analysis previously revealed a distinct metabolic signature for HNPGGLs, in particular, the importance of lysophosphatidylcholines, glutamate, deoxyadenosine, and some long-chain acylcarnitines as potential biomarkers of HNPGGL. In the current study, we confirmed the pivotal role of glutamine (Gln) in tumor metabolism as an anaplerotic substrate as well as metabolism involved in the urea cycle (Arginine). Interestingly, we found a characteristic signature of medium-chain acylcarnitines in GBM/LGG plasma samples compared to healthy controls suggesting an alteration of mitochondrial fatty acid β -oxidation. Ultimately, a multivariate analysis based on ROC curves is conducted to establish a predictive model utilizing metabolite levels.

CONCLUSIONS

Targeted FIA-MS/MS, which allows for the assessment of clinically important compounds in plasma or dried blood spots, could provide an effective screening tool for these tumors and could contribute to our understanding of the metabolic pathways underlying neural crest cancers.

THE ANTHELMINTIC MEBENDAZOLE IS A PROMISING CANDIDATE FOR REPURPOSING IN PANCREATIC CANCER TREATMENT

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BACKGROUND-AIM

Pancreatic cancer (PC) is one of the most challenging tumors. Standard chemotherapy is currently unsatisfactory, thus novel therapeutic options are needed. In this regard, drug repurposing is a promising approach to improve PC therapy. Mebendazole (MBZ) is an anthelmintic drug that showed to be active in several cell and animal cancer models. Here, we investigated the antitumor effects of MBZ in different preclinical models of PC.

METHODS

Antiproliferative effects of MBZ were evaluated in five PC cell lines (AsPC-1, BxPC-3, Capan-1, L36PI, PATU8902) with distinct genetic profiles. Viability and self-renewal capacity of PC cells after treatment were assessed by MTT and clonogenic assays, respectively. Flow cytometry and immunoblot analyses were used for evaluating apoptosis and cell cycle distribution in PC cells treated with MBZ. Antitumor potential of MBZ was evaluated in NSG mice, xenografted with L36PI cells. At the end of the experiments, mice were euthanized, organs and tumors were analyzed to evaluate toxicity and efficacy of MBZ.

RESULTS

Our results revealed a PC cell-dependent response to MBZ. The drug markedly and consistently inhibited viability and clonogenicity in BxPC-3, L36PI and PATU8902 cells, with good selectivity indexes compared to normal HFF-1 cells, whereas AsPC-1 and Capan-1 were more resistant to MBZ. Mechanistically, apoptosis and DNA damage appeared to contribute to the reduced PC cell viability induced by MBZ, as indicated by flow cytometry evaluation of Annexin-V and immunoblot analysis of PARP cleavage. In addition, MBZ profoundly interfered with PC cell cycle progression, although with a certain degree of heterogeneity across PC cell lines, as indicated by flow cytometry and alteration of the expression of cell cycle proteins. In vivo, at 30 mg/kg MBZ was well tolerated and significantly inhibited pancreatic tumor growth compared to control group.

CONCLUSIONS

These preliminary results provide valuable insights supporting the potential repurposing of MBZ in PC treatment. Supported by Unione Europea – NextGenerationEU, "MUR-Fondo Promozione e Sviluppo - DM 737/2021", PDAC-TREAT: Testing repurposed drugs candidates in pancreatic ductal adenocarcinomas: a multilevel in vitro/in vivo approach for improved therapy.

LIQUID BIOPSY IN ONCOLOGY: ANALYSIS OF RAS AND BRAF GENES MUTATIONAL PROFILE IN METASTATIC COLORECTAL CANCER PATIENTS.

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BACKGROUND-AIM

Metastatic colorectal cancer (mCRC) is characterized by a high frequency of KRAS mutations and BRAF mutations at the valine 600 residue are also associated to poor prognosis due to the limited response to EGFR inhibitors (Van Cutsem E et al., 2011). According to the ESMO guidelines patients are screened for KRAS, NRAS and BRAF to select the most appropriate to be treated with anti-EGFR (Van Cutsem E et al., 2016). The aim of this work is to better decipher the molecular background of mCRC and to exploit it as a tool for personalized medicine.

METHODS

K- and NRAS status were determined on tissue by NGS and by MALDI-TOF. 8ml of peripheral blood for ctDNA analysis were collected from 62 mCRC patients before first-line treatment and 4-8-12 weeks after starting treatment until disease progression. Plasma was used for the determination of KRAS, NRAS and BRAF status using either OncoBEAM® RAS CRC assay (Sysmex Inostics, Hamburg, Germany) and Idylla™ ctKRAS/ctNRAS-BRAF Mutation Test (Biocartis, Mechelen, Belgium).

RESULTS

66.1% of the patients harbored KRAS mutations in FFPE tumor tissue biopsies while only one patient (1.6%) showed NRAS mutation. Only 9.8% of the patients presented BRAF mutations. In plasma, the most represented mutations were on KRAS codon 12, followed by codon 13 and 146. As concerning NRAS, 12 patients had mutations on codon 12 and 61. Cohen's K revealed a good concordance between OncoBEAM and tissue analysis for both KRAS and NRAS; Idylla had a perfect and substantial concordance with tissue analysis for NRAS and BRAF, respectively. Also, a moderate concordance emerged comparing the type of mutation with tissue analysis and Idylla. Interestingly, significantly higher Mutant Allele Fraction (MAF) levels were detected in patients with G2 tumors, liver metastases, and in those who did not receive surgery. NRAS MAF level was significantly higher in patients with mucinous adenocarcinoma and for those with lung metastases. A sharp increase in the MAF values was observed in patients whose disease progressed. More strikingly, molecular progression always anticipated the radiological one in these patients.

CONCLUSIONS

Our data suggest the possibility of using liquid biopsy to monitor patients during treatment, and to enable oncologists to anticipate interventions compared to radiological analyses.

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A NOTCH INHIBITOR PLUS RESVERATROL DOWNREGULATED THE CD1/CDK4 SIGNALING SHAPING THE PHENOTYPE OF TRIPLE-NEGATIVE BREAST CANCER CELLS

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is characterized by variations in histology, genomics, and immunological features. Through the utilization of advanced sequencing and omics techniques, the extensive heterogeneity within TNBC has been unveiled, providing insights into the underlying mechanisms and therapeutic complexities of this subtype of breast cancer. Aberrant activation of the Notch signaling pathway has been strongly implicated in the development and pathogenesis of TNBC. The gamma-secretase inhibitor RO4929097 (GSI) exerts potent inhibitory effects on Notch signaling. The use of these therapeutic agents has faced limitations due to their inhibitory effects on other signaling pathways and the occurrence of severe intestinal toxicity in cancer patients. Conversely, combined approaches have shown greater efficacy. One such potential candidate is Resveratrol (RSV), which has demonstrated antiproliferative properties and a protective effect against various types of cancers.

METHODS

MTT assay, Western Blotting assay, Wound Healing Scratch assay, Invasion assay.

RESULTS

Combined treatment with low doses of RSV and GSI, reduced the expression levels of cyclin D1 and its binding partner CDK4 and increased p27 in TNBC cells, instead p-AKT and PTEN unchanged. Wound Healing Scratch assay showed that cells co-treated with RSV and GSI, move much slower to close the gap compared with control or single treatments. The ability of these cells to cross and invade a microporous membrane, was evaluated through Invasion assay. Co-treated cells showed a reduced invasive phenotype compared to untreated control cells. Moreover, slowdown of cell proliferation was observed after co-treatment.

CONCLUSIONS

The study emphasizes the inhibitory effects of the combined treatment with RSV and GSI on the overexpression of cyclin D1 and its binding partner CDK4. This combined approach has the potential to reduce the motility and invasiveness of breast cancer cells. By targeting these key molecules, the combination therapy acts to disrupt pathways associated with TNBC cells aggressiveness, to mitigate their invasive potential.

EVIDENCE OF ANTI-INFLAMMATORY ACTIVITY OF CANNABINOIDS ON MONOCYTE/MACROPHAGE CELL LINE THP-1

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BACKGROUND-AIM

Several studies have demonstrated the ability of cannabinoids to suppress inflammatory response, reducing the expression of pro-inflammatory cytokines and increasing levels of anti-inflammatory cytokines. In order to study the mechanisms related to immunomodulation triggered by cannabinoids, this study analyzes the anti-inflammatory ability of three non-psychoactive component of Cannabis sativa, cannabidiol (CBD), cannabidiolic acid (CBDA) and cannabigerol (CBG), to influence immunocompetent cells by using THP-1, an established monocytic leukemic lineage, able to differentiate into macrophages after treatment of PMA.

METHODS

THP-1 cells were pre-activated with PMA (1 µg/mL) for 3 days. The differentiated macrophages obtained were pre-incubated with CBD 0.3 µM and 1 µM for 1h, and subsequently, cells were treated with LPS (5µg/mL) for 24 and 48h, respectively, in order to stimulate inflammatory response. Supernatants were then analyzed using the ELISA assay. The most effective concentration of CBD (1µM/mL) were compared with the effectiveness of the same concentration of CBG and CBDA in the 24h.

RESULTS

As expected LPS treated macrophages increased their pro-inflammatory IL-6 expression. Pre-treating cells with CBD reduce the IL-6 expression. Cells treated with 1 µM CBD expressed less IL-6 than cells treated with CBD 0.3 µM, suggesting that the anti-inflammatory ability of CBD is dose-dependent. CBDA was the most effective for reducing the IL-6 expression in the 24h, followed by CBG and CBD.

CONCLUSIONS

All the cannabinoids tested, CBD, CBDA and CBG, showed the ability to reduce IL-6 expression in the following 24 hours, even if the most efficient anti-inflammatory compound was CBDA. As a consequence, our findings confirmed the anti-inflammatory ability of these compounds, suggesting that they may have therapeutic potential for inflammatory diseases. Further studies are currently underway.

ANDROGEN ACTION IN COLON CANCER

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BACKGROUND-AIM

Colorectal cancer is the third most common cancer and represents the second leading cause of cancer-related deaths worldwide. CRC shows a higher incidence and a worse survival outcome in men than in women and this difference is markedly cleared in every region of the world by the analysis of age-standardized incidence rates. These data support scientists to deepen their knowledge about the mechanisms controlled by the androgen receptor (AR) in CRC. The role of AR is well known in prostate cancer but different data shows that, through genomic or non-genomic actions, it can influence growth and invasiveness as well as epithelial to mesenchymal transitions (EMT) even in other cancers such as breast, melanoma, pancreas, bladder.

METHODS

In this study, we have used CRC-derived Caco2, LoVo and HCT-116 cells, expressing AR at different levels. BrdU incorporation and wound healing assays show that androgen challenge induces invasion and proliferation of these cells. The antiandrogen Bicalutamide or two small peptides, S1 and Rh2025u designed to mimic the AR sequences responsible for the interaction of the receptor with Src or Filamin A, respectively, reverse the androgen-induced effects in all the cell lines at different extend. Biochemical approaches show that the androgen treatment induces the association between AR and other partners such as Filamin A or src and the activation of different effectors.

RESULTS

Our findings demonstrate that AR specifically interacts with different partners in androgen-treated CRC cells. These associations could be responsible for the activation of Rac, PKC, and other proteins thereby controlling the proliferation and migration of different CRC-derived cells.

CONCLUSIONS

The present study emphasizes the role of AR in CRC and highlights how this receptor could contribute to CRC onset and progression, especially in patients with altered concentrations of androgens. Clarifying the role of this steroid receptor in colon cancer can pave the way to developing new screening campaigns and new specific and effective therapies such as use of the antiandrogens or AR-targeting peptides.

REGENERATIVE NANOMEDICINE: DUAL STEM CELL-BASED THERAPY POTENTIATED BY PROANGIOGENIC GOLD NANOPARTICLE DELIVERY FOR TISSUE VASCULARIZATION

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BACKGROUND-AIM

Cardiovascular disease (CVD) is a group of cardiac and circulatory diseases considered the first cause of death worldwide. Novel approaches promoting neovascularization are urgently required to increase the therapeutic effects and reduce the surgery-related risk burden on patients.

Both Mesenchymal stem cells (MSCs) and Endothelial colony-forming cells (ECFCs) hold significant promise as candidates for regenerative cell therapy of vascular injury: ECFCs because of their high clonogenic potential and ability to originate de novo blood vessels in vivo, MSCs due to their ability to differentiate into cardiovascular cells, antifibrotic activity and ability to undergo neo-vasculogenesis.

Despite nanomedicine is considered a novel and effective approach for targeted vascular disease treatment, its application in CVD is still challenging. The difficulty lies in the delivery of nanoparticles to specific blood vessel lesions. Since cotransplanting ECFCs into the grafts provides sufficient trophic support to MSCs, we hypothesized that MSC and ECFC coculture, used also as cargo of proangiogenic gold nanoparticles (AuNPs) tested in our laboratory, could potentiate the regenerative therapeutic effect of each one.

METHODS

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

RESULTS

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

CONCLUSIONS

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



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