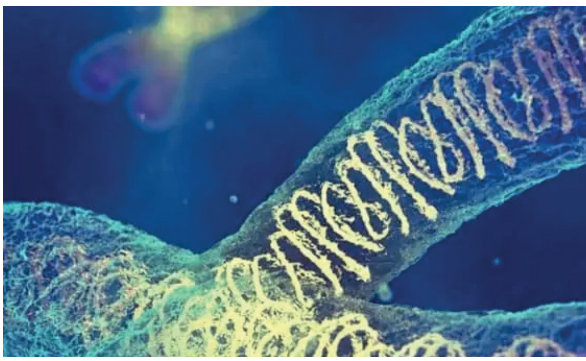
ABSTRACT BOOK

SIPMeT Young Scientist Meeting

MOLECULAR PATHOLOGY: FROM BENCH TO BEDSIDE

December 10 - 11, 2021

Aula Magna Rettorato, Università degli Studi di Perugia, Perugia



P001

GLUTAMINE DEPENDENCY IN COLORECTAL CANCER CELLS: A METABOLOMICS STUDY

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BACKGROUND-AIM

Cancer cells adapt their metabolism to meet energy demand. Some nutrients become essential for tumour growth and progression and their deprivation alters the bioenergetic profile of cancer cells, leading to proliferation arrest or adaptation. Glutamine is considered a "conditionally essential" aminoacid and plays a key role in cancer cell metabolism. Indeed, glutamine addiction represents a distinctive feature of several types of tumour, as colorectal cancer. Regarding clinical practice, identifying which tumours are susceptible to glutamine deprivation represents a useful challenge to improve targeted therapeutic strategies. In this perspective, metabolomics is a promising approach to investigate pathophysiological aspects of cancer metabolism and to find new potential biomarkers and therapeutic targets.

METHODS

In the present study, 4 colorectal cancer cell lines (Caco-2, HCT116, HT29 and SW480) were cultured in complete medium (DMEM high glucose) with or without glutamine (4mM). Growth rate and proliferation capacity were evaluated by MTT (4 days) and the colony forming assay (14 days), respectively. Redox homeostasis was performed by measuring aminothyol levels (reduced and oxidized glutathione, GSH and GSSG), through HPLC analysis. Metabolomics analysis was conducted with Gas-Chromatography-Mass-Spectrometry and Multivariate and Univariate Statistical approaches to identify metabolic alterations related to glutamine deprivation.

RESULTS

Glutamine deprivation altered the cell growth rate and proliferation capacity. A significant decrease of the growth rate (~50%) was observed in all cell lines. Moreover, glutamine starvation leads to a considerable decrease in antioxidant species, expressed as GSH/GSSH ratio. From the metabolomics point of view, multivariate models showed significant metabolic differences of the groups based on glutamine starvation. In particular, changes in metabolites concentration correlated to glutathione metabolism (glycine, 5-oxoproline), amino acid pathways (serine, threonine) and energetic processes (glucose, galactose) were observed.

CONCLUSIONS

This study represents an intriguingly starting point for better understanding the key role of glutamine pathway in cancer metabolism and to identify potential biomarkers useful to improve targeted therapies.

P002

GADD45B AS A POTENTIAL THERAPEUTIC TARGET IN ACUTE MYELOID LEUKEMIA

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BACKGROUND-AIM

Acute myeloid leukemia (AML) is a highly heterogeneous disease and is the second most common form of leukemia, accounting for about a third of adult cases. Virtually, all cases of AML display elevated nuclear NF-κB activity. This aberrant NF-κB activity drives stem-cell survival, self-renewal and therapy resistance, leading to relapse. However, no specific NF-κB inhibitors has been clinically approved, due to the dose-limiting toxicities caused by the general suppression of NF-κB. GADD45B is an important mediator of the cytoprotective/anti-apoptotic activity of NF-κB and it was demonstrated to be an alternative therapeutic target in multiple myeloma. Given the relationship between NF-κB and GADD45B, we went to investigate the possible role of this protein in AML.

METHODS

We performed a bioinformatic analysis on a public AML dataset characterizing the distribution and the expression of GADD45B in AML patients, as well as the correlation between NF-κB target-gene and GADD45B expression. Western Blot analysis, qRT-PCR and Cell-Titer Glo viability assays were performed to assess the role of GADD45B and therapeutic efficacy of anti-GADD45B agents in a panel of AML cell lines.

RESULTS

Our preliminary data suggest that GADD45B mediates NF-κB-dependent malignant cell survival in AML. Our analysis of patient datasets demonstrated the wide distribution and overall high expression of GADD45B in AML. GADD45B expression was significantly higher in M3, M4 and M5 AML than other FAB subtypes and strongly correlated with the inflammatory and NF-κB target-gene signatures, suggesting a role for GADD45B in NF-κB-driven AML pathogenesis. Congruently, DTP3 displayed a potent capacity to kill AML cell lines exhibiting elevated GADD45B expression.

CONCLUSIONS

These findings suggest that anti-GADD45B agents could be effective for treating discrete AML subsets, thereby providing a strong rationale for developing these agents in AML.

P003

INHIBITING THE AXIS NF-κB/GLI1 IN PROSTATE CANCER AS A POTENTIAL THERAPEUTIC STRATEGY

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BACKGROUND-AIM

Prostate cancer (PCa) is the second most frequent cancer in men worldwide. NF-κB seems to play a key role in cell survival, proliferation and invasion sustaining the heterogeneous multifocal nature of PCa. Over the recent years, Hedgehog (Hh) signalling pathway attracted attention as therapeutic target due to its implication in tumorigenesis and metastasis in several type of cancer including PCa. Although it is well-known that Sonic Hedgehog (SHh) is a transcriptional target of NF-κB and GLI1 is the effector of this crosstalk, the precise role played by this axis within the PCa is still not completely clear.

METHODS

We used TCGA Prostatic Cancer (PRAD) dataset to analyze the expression of RELA, SHh and GLI1 as well as the survival of PCa patients and the gene signature expression. Molecular analyses were performed to study the co-expression for NF-κB, SHh and GLI1 in advanced PCa using human cell lines and tissue samples.

RESULTS

Our findings demonstrated a relevant co-expression for NF-κB, SHh and GLI1 in advanced PCa samples, highlighting a positive interplay between these pathways in aggressive PCa disease and suggesting that targeting this crosstalk could be a successful strategy to treat advanced PCa showing NF-κB and GLI1 activation.

CONCLUSIONS

This study represents a new step forward for the understanding of NF-κB/SHh interplay in PCa and provides a novel therapeutic option for the treatment of PCa patients with constitutive NF-κB and GLI1 activation.

P004

DECODING POST-TRANSLATIONAL MODIFICATIONS OF NOTCH3 INVOLVED IN PROTEIN ACTIVITY AND STABILITY

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BACKGROUND-AIM

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

METHODS

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

RESULTS

Firstly, we evaluated which amminoiacids are responsible for the interaction between Pin1 and N3 by MS and protein conformational analysis. Moreover, we documented that Pin1 sustains the expression of N3 intracellular domain by preventing its proteasomal degradation, promoted by the GSK3β kinase/E3-ligase WWP2 intercrossed activity. Finally, we investigated the potential relevance of the Pin1/N3 cross-talk in escaping from this negative regulation process in OC context through the evaluation of the N3 protein stability and ubiquitination status.

CONCLUSIONS

Our findings demostrated the antagonistic effect of Pin1 and GSK3β on N3, thus acting one as a positive regulator (Pin1) and the other one as negative regulator (GSK3β), and suggested WWP2 as another potential negative regulator involved in Pin1-GSK3β-N3 axis.

P005

NAT10 INVOLVEMENT IN MYC REGULATION BY EPIGENETIC DRUGS

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BACKGROUND-AIM

Tumor suppressor p53 has a key role in regulation of several genes, including the oncogene MYC. p53 and MYC interact in a negative crosstalk required to ensure tissue homeostasis (Sachdeva et al, 2009). MYC is a hallmark of malignant growth, causing stem cells self-renewal and blocking cell death and differentiation. Therefore, MYC is an attractive target for anticancer therapy. However, lacking enzymatic activity and deep targetable pockets MYC is not druggable protein: MYC co-factors inhibition and/or MYC repressors could represent a valid strategy to bypass this limit (Scafuro et al, 2021). We already demonstrated that HDAC inhibitors hyperacetylate and down-regulate MYC, blocking cancer proliferation (Nebbioso et al, 2017). MYC can be a useful biomarker for HDACi responsiveness. NAT10 is an acetyltransferase able to modulate p53, by acetylating K120 residue. Thus post-translational mdifications modulate p53 and its target genes, including MYC.
We aim to identify a target easily druggable by epidrugs within MYC/p53 transduction signal.

METHODS

Human acute myeloid leukaemia (OCI-AML3) and colon cancer (HCT116) cell lines, wildtype for p53, are treated with the HDACi SAHA and the NAT10 inhibitor Remodelin and studied by western blot (WB), qPCR and immunofluorescence (IF) analyses.

RESULTS

To identify the HAT involved in SAHA-mediated MYC hyperacetylation, we investigated NAT10 role as potential HDACi target, able to modulate MYC activity within MYC/p53 transduction signal. NAT10 has been reported to form micronuclei (MN) during nucleosomes maturation by acetylating histones (Cao et al, 2020). In both our cell lines SAHA upregulates and concentrates NAT10 into MN (WB, qPCR and IF). MN staining is disappeared by Remodelin, supporting an effect of SAHA on NAT10. Notably, Remodelin blocks SAHA-medited MYC hyperacetylation, leading to MYC hyperexpression. To better understand how HDAC inhibition induces a loss af oncogenic activity of MYC, we study its cell localization: MYC translocates in cytoplasm in cells treated with SAHA while it remains into nuclei with remodelin. Futher investigations are required and ongoing to elucidate NAT10 involvment in MYC regulation by epidrugs.

CONCLUSIONS

Our results show NAT10 as a potential biomarker of HDACi antitumor action in MYC/p53 signalling pathways

P006

ANTI-INFLAMMATORY EFFECT OF OLIVE LEAF EXTRACT AND ITS ACTIVE COMPOUNDS: A POTENTIAL APPROACH AGAINST COVID-19?

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BACKGROUND-AIM

The SARS-CoV2 virus has caused a sudden global alarm due to the potential mortality provoked by its associated disease. This clinical picture is not only caused by viral infection itself, but also by the intense inflammatory response of the host, which, in the final stage becomes uncontrolled and is characterized by the so-called cytokine storm.
In recent years, the anti-inflammatory properties of natural compounds have aroused enormous interest among the scientific community. Olive leaves are a source of many phytochemicals like phenolics and flavonoids. Moreover, they represent a waste by-product for olive oil sector, therefore readily available at low cost. Here we have investigated their possible ability to modulate the inflammatory response of the human cells which are mainly involved in the response against Sars-CoV2 Infection.
The results presented represent part of the objectives of the FISR2020 OLEA-ACT project

METHODS

Fresh olive leaves were dried and grinded to obtain a powder, which was macerated in PBS. Total phenolic content (TPC) and composition of the Olive Leaf Extract (OLE) were analysed by Folin-Ciocalteu reagent and HPLC method respectively. OLE and two of its components, oleacein (OC) and Oleuropein aglycone (OA), were tested for anti-inflammatory activity in LPS-stimulated young human umbilical vein endothelial cells (HUVECs) and human monocyte cell line (THP-1) and furthermore in senescent HUVECs. Cytotoxicity was assessed by MTT assay. RT-PCR, Western Blot and ELISA assay were used to analyse the expression of NF-kB, pro-inflammatory cytokines and adhesion molecules

RESULTS

In LPS-stimulated THP-1 pre-treatment with OLE or OA or OC inhibited significantly NF-kB, IL-1β, IL-6 and TNF-α and IL-8 production. Interestingly, OLE seems to have a better effect than the single compounds. Similar results were also observed in LPS-treated young HUVECs and senescent HUVECs where we also observed a decrease in the expression of adhesion molecules (ICAM and VCAM)

CONCLUSIONS

OLE and its component poorly studied, OA and OC, can exert an anti-inflammatory effect in in vitro model of acute and senescence related inflammation thus suggesting their use as a possible approach against Covid-19 cytokine storm

P007

(2S,4R)-4-FLUOROGLUTAMINE IS CONVERTED INTO (2S,4R)-4-FLUOROGLUTAMATE BY GLUTAMINASE ACTIVITY AND MAY BE EXPLOITED AS A PET TRACER IN GLUTAMINE-ADDICTED CANCERS

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BACKGROUND-AIM

Glutamine (Gln)-addicted tumors meet their metabolic needs markedly increasing Gln uptake and glutaminase (GLS)-dependent Gln utilization. These cancers are sensitive to GLS inhibitors and novel methods for their identification are thus badly needed. In cooperation with the San Raffaele Institute (Dept of Nuclear Medicine and of Immunology) we have recently demonstrated that Gln-addiction of multiple myeloma (MM) (Bolzoni et al, Blood 2016; Chiu et al, Cancers 2020) allows the exploitation of [^{18}F](2S,4R)-4-fluoroglutamine ([^{18}F]-Gln) as a PET tracer in murine models of this tumor. Compared to [^{18}F]fluorodeoxyglucose (FDG), [^{18}F]-Gln exhibits higher sensitivity in detecting bortezomib effects (Valtorta et al, Front. Oncol. In press). To better evaluate the potential of [^{18}F]-Gln as a PET tracer in MM, we have assessed here its ability to be a GLS substrate and, hence, to detect high-GLS cancers.

METHODS

Enantiopure (2S,4R)-4-fluoroglutamine (F-Gln) has been synthesized and its uptake compared with that of Gln in a panel of human cancer cell lines: RPMI8226 and JJN3 (MM), U2OS and HOS (osteosarcoma), Huh7 (hepatocellular carcinoma), PANC (pancreatic adenocarcinoma). The expression of GLS was assessed in parallel. Intracellular levels of F-Gln and F-Glu were measured by HILIC liquid chromatography coupled to tandem mass spectrometry.

RESULTS

F-Gln was rapidly accumulated by cancer cells and inhibited Gln uptake with a potency comparable to that of the natural amino acid. Moreover, F-Gln and Gln uptakes were sensitive to the same transporter-specific inhibitors. Upon incubation with F-Gln, F-Glu was produced, and its content significantly correlated with GLS expression in the cell lines tested. GLS inhibition or silencing impaired F-Glu production.

CONCLUSIONS

F-Gln exploits the same transporters of Gln and is converted into F-Glu by GLS. The amount of F-Glu produced is therefore an indicator of GLS activity, suggesting that, in vivo, the retention of [^{18}F]-Gln/Glu may be higher in Gln-addicted, high-GLS cancers. These data suggest that F-Gln may be a tool to investigate GLS activity in intact cells and strengthen the potential of [^{18}F](2S,4R)-4-fluoroglutamine as a novel PET tracer for the follow up of Gln-addicted, high-GLS MM.

P008

AEROBIC GLYCOLYSIS AND ACTIVE CITRATE SECRETION ARE METABOLIC FEATURES OF BONE MARROW MESENCHYMAL STEM CELLS UNDER PHYSIOLOGICAL CONDITIONS

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BACKGROUND-AIM

Bone marrow (BM) mesenchymal stem cells (MSCs) can differentiate into adipocytes, chondroblasts and osteoblasts, migrate towards the sites of injury, modulate immune response and sustain hemopoiesis. For these reasons, MSCs are increasingly used in regenerative medicine and in advanced immunosuppressive therapies. Recently, our group demonstrated that MSCs, through amino acid cross-talks, play important roles in haematological cancers, supporting malignant blasts in acute lymphoblastic leukaemia (Blood Advances, in press) and contributing to osteolytic lesions in multiple myeloma (Cancers, 2021). However, the metabolic profile of MSCs remains to be fully elucidated.

METHODS

We have evaluated transcriptome (RNAseq) and the metabolic profile (untargeted and targeted LC-MS, $^{13}C_6$ Glucose, $^{13}C_5$ Glutamine) in primary MSC strains derived from different donors (n = 8). Cells have been cultured in DMEM or in Plasmax™, an advanced physiological plasma-like medium.

RESULTS

Intracellular metabolic profile and exchange rates of MSCs were significantly different in Plasmax and DMEM, where cells underwent severe nutritional stress. At 21% O_2 in Plasmax, more than 60% of the glucose consumed (20 nmol/µg/day) was converted into lactate, while almost 50% of the glutamine consumed (> 3 nmol/µg/day) was used for anaplerosis. Unexpectedly, citrate was actively secreted by all the cell strains at an average rate of 1.5 nmol/µg/day. At 1% O_2, MSCs increased glycolytic flux, reductive carboxylation of glutamine-derived 2-oxoglutarate and glutamate consumption and still secreted citrate, although at a reduced rate.

CONCLUSIONS

These results indicate that (a) standard cell culture media can severely skew the metabolic profile of cultured cells; (b) although they are normal diploid cells, MSCs exhibit high glycolytic flux and glutamine consumption at high O_2, similar to a cancer/pluripotent-like metabolic phenotype; (c) MSCs actively secrete citrate, pointing to a role for the tricarboxylic acid in the hemopoietic niche. The metabolic characterization of MSCs under physiological conditions lays the basis for more comprehensive studies on stromal contribution to normal and neoplastic hemopoiesis.

P009

LEUCOCYTE CELL POPULATION DATA IN SARS COV-2 INFECTED PATIENTS ADMITTED TO A CARDIOLOGY HOSPITAL. CELLULAR ANOMALIES LINKED TO MOLECULAR IMBALANCES

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BACKGROUND-AIM

It is well known that high values of leucocyte cell population data (CPD) occur in sepsis. Hematological alterations during Sars Cov-2 infection (Covid-19) were well described in term of cell count reduction such as lymphopenia and thrombocytopenia. In the present study, CPD values of patients admitted to a cardiology hospital were analyzed.

METHODS

During the first outbreak, we evaluated 540 patients divided into: 490 patients without signs of Covid-19 (reference population) and 50 patients Covid-19 positive confirmed by molecular testing (nucleic acid amplification test) (NAAT). Along with biochemical and clinical data have been collected hematological parameters: RBC, PLT and WBC with subpopulations: neutrophils, lymphocytes and monocytes. We retrospectively evaluated as optical signals the following CPD parameters: NE-SSC, NESFL, NEFSC, LY-X, LY-Y, LY-Z, MO-X, MO-Y, MO-Z, NE-WX, NE-WY, NE-WZ, LY-WZ, LY-WY, LY-WZ, MO-WX, MO-WY, MO-WZ, obtained by a XN analyzer (Sysmex Corporation, Kobe, Japan). A statistical analysis using general linear models adjusted for age and sex was performed.

RESULTS

Among 18 CPD values, 9 parameters (50%) were significantly increased compared to those of the reference population as follows: (NE-SFL)(ch) (p=0.0116), (LY-X)(ch) (p= 0.001), (LY-Z)(ch) (p= <0.001), (MO-X)(ch) (p=<0.001), (MO-Y)(ch) (p=0.0206), (NE-WX)(ch) (p=0.0002), (NE-WY)(ch) (p=0.0323), (NE-WZ)(ch) (p=0.0037), (MO-WX)(ch) (p=0.0054). All parameters were significantly confirmed even after adjustment of confounder factors. Of 50 patients Covid-19 positive, 38 (76%) developed Sar Cov-2 pneumonia. Of which, along with hematological abnormalities, patients recorded pathologic C-reactive protein (CRP) and/or lactate dehydrogenase (LDH) levels.

CONCLUSIONS

We observed a relationship between metabolic derangements during Sars Cov-2 infection and morphological/ functional characteristics of leucocytes. Most CPDs showed abnormalities in cell complexity and DNA/RNA cell content.

P010

GLABRESCIONE B ENCAPSULATED IN SELF-ASSEMBLING MICELLES INHIBITS TUMOR GROWTH IN PRECLINICAL MODELS OF HEDGEHOG-DEPENDENT MEDULLOBLASTOMA

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BACKGROUND-AIM

The Hedgehog (Hh) signalling pathway plays a crucial role in development and tumorigenesis and is emerged as promising target for several tumors, including medulloblastoma (MB).
Glabrescione B (GlaB) is a natural compound and the first small molecule able to directly inhibit Gli1, the most powerful effector of the Hh pathway. GlaB represents a promising molecule with great anti-tumor properties that stands as a good candidate drug for pre-clinical studies in the treatment of Hh-dependent tumors

METHODS

In order to overcome the poor water solubility of GlaB and to enhance its therapeutic efficacy, several dissolution strategies have been explored. The toxicity, delivery, biodistribution, and anticancer efficacy of GlaB encapsulated in micelle forming polymers were investigated in preclinical models of Hh-dependent MB.

RESULTS

Our studies show that the amphiphilic polymer mPEG5kDa-cholane enhances the solubility of GlaB, unlike commercially available surfactants, avoiding the use of organic solvents. In virtue of its physic-chemical properties, mPEG5kDa-cholane undergoes self-assembling in spherical micellar colloidal systems that encapsulate GlaB in confined supramolecular structures, by promoting its solubility and preserving its chemical identity. mPEG5kDa-cholane/GlaB shows high drug loading and stability, low cytotoxicity, and long permanence in the bloodstream. Thanks to these properties, GlaB formulated in mPEG5kDa-cholane affects the in vitro proliferation of primary Hh-MB cells and significantly impairs Gli1 transcriptional activity compared to free GlaB.
Remarkably, GlaB encapsulated in mPEG5kDa-cholane micelles was delivered through the blood-brain barrier and drastically inhibited tumor growth in both allograft and orthotopic models of Hh-dependent MB. In agreement with these results, GlaB encapsulated in mPEG5kDa-cholane has a greater effect in inhibiting tumor growth in both allograft and orthotopic models of Hh-dependent MB when compared to free GlaB, by improving its effectiveness at lower concentrations.

CONCLUSIONS

Our findings reveal the excellent anti-tumor properties of mPEG5kDa-cholane/GlaB formulation for the treatment of Hh-driven tumors and provides relevant implications for the translation of GlaB into clinical practice.

P011

WASTE PROTEINS AS A RESOURCE: CROSSTALK BETWEEN EPIGENETIC AND AUTOPHAGY IN RESISTANT BREAST CANCER

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BACKGROUND-AIM

Autophagy is a cellular homeostasis mechanism activated for the degradation of misfolded proteins and damaged organelles to maintain cell metabolism. In physiological condition it guarantees metabolic substrates through recycle of macromolecules. Autophagy is negatively linked to many diseases, including cancer. Indeed, it plays a dual role in cell survival and cell death in context/time dependent, blocking cancer progression by suppressing tumor initiation in early stages and by promoting survival and grow of tumor cells in late stages. It is also considered as a defense mechanism to chemotherapy: it allows cancer cells to resist the damage by drugs, inducing resistance to anticancer treatments. The project aims to determine the role of autophagy in BC sensible and resistant to endocrine therapy, to evaluate if autophagy can be a therapeutic target combined with drugs and how this process can be regulated combining the epigenetic treatment with autophagic modulators.

METHODS

Differential analysis between MCF7 and MCF7/Tam resistant cells would allow us to clarify if and how autophagy can affect the mechanism resistance.

RESULTS

Preliminary results showed higher basal levels of autophagy in MCF7/TamR compared to MCF7. These data were demonstrated by analyzing expression of proteins involved in autophagic pathway revealing an increase of basal levels of LC3B, protein associated with completed autophagosomes, and a decrease of p62 delivering ubiquitinated proteins to the proteasome for degradation. Degradation of p62 is based on the activity of HDAC6, playing a pivotal role in aggresome formation. To evaluate if autophagy can be a therapeutic target combined with drugs and how it can be regulated, we co-treated cells with chemotherapeutics and modulators of autophagy, evaluating the different responsiveness to treatments. Our findings suggest a critical role of autophagy in BC cells, sensible and resistant to endocrine therapy.

CONCLUSIONS

Under chemotherapy-stressed conditions, the cells activate autophagic defence rendering them more resistant to the anticancer effects of drug. The choice of a selective epidrug to block autophagic flux in combination and or as adjuvant therapy can represent a precise target to enhance tumor cell chemosensitivity thus promoting cell death.

P012

ALTERNATIVE SPLICING AND CANCER EPIGENETIC: BCLAF1 ROLE IN COLORECTAL CANCER

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BACKGROUND-AIM

Bcl-2-associated transcription factor 1 (BCLAF1) is confirmed that has a multiple role in different cellular processes such as cell developments and immune system functional regulation, etc. It has been reported that BCLAF1 is able to induce the angiogenesis through upregulation of HIF-1α by binding to the target gene promoter. BCLAF1 is an important regulator of PD-L1 and is demonstrated that BCLAF1 knock down induce reduction in PD-L1 expression through the ubiquitination of this immune checkpoint protein. In AML, miRNA-194-5p can downregulate BCLAF1 expression in posttranscriptional manner by binding to the BCLAF1 3'-UTR. In 2014, it was reported that there is a precise splicing isoform of BCLAF1 in CRC including exone5a that significantly increase in CRC samples. Our aim is the molecular and functional characterization of these isoforms in two CRC modals. Furthermore, we investigate the epigenetic regulation of epi-drugs on the BCLAF1 alternative splicing machinery.

METHODS

Cell Culture, QRT-PCR, Western blot analysis, Human tissue microarray (TMA), comprehensive statistical meta-analysis

RESULTS

We confirmed the overexpression of BCLAF1 expression in CRC cell lines and primary samples, compared with healthy control. In addition, we correlated its higher expression with metastatic potential, the right colon site and the prominent vascular infiltration of primary CRC tumors.
We characterized the potential SAHA to reduce selectivity the BCLAF1 full length isoform and increase –exon5a one. In addition, SRSF10 gene expression showed the same pattern of BCLAF1 expression in HCT116wt after SAHA treatment, however in DKO SRSF10 did not affected by the drug. We hypnotized that in DKO cells, BCLAF1 gene expression can be regulated by other regulatory mechanism instead of SRSF10. Also, the observed BCALF1 isoform induced by SAHA might be an evidence to unravelling the specific loop between BCLAF1 alternative splicing and the methylome machinery which can be DNA or RNA methylation system. Different HDAC inhibitors effect on the expression of BCLAF1 and SRSF10 have been analyzed, both in cell line and primary material.

CONCLUSIONS

These finding can make a new bridge in unravelling the correlation of alternative splicing and cancer epigenetic in the CRC.

P013

A NEW KEY TO UNDERSTAND GENDER DISPARITY IN MELANOMA PROGRESSION: ESTROGEN RECEPTOR BETA EXPRESSION IN THE ACIDIC MICROENVIRONMENT

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BACKGROUND-AIM

Melanoma incidence is increasing, but prognostic factors available for melanoma development and progression remain poorly known. Among these factors, the most intriguing is sex: men have a higher risk of developing this cancer than women, and a worse prognosis if they do. Researchers suggest that there is a biological trait not yet fully elucidated, which accounts for the sex-related survival advantage in melanoma and some evidence supports a correlation between sex hormones and melanoma progression. Estrogens exert their effects through estrogen receptors among which ERß plays a major role.
The objective of this study is to elucidate the mechanisms underlying the sex disparity observed in melanoma progression considering cell plasticity and ERß expression. In order to test whether ERß expression is a specific characteristic of a gender phenotype; melanoma cells were exposed to an acidic medium to mimic the acidosis of tumor microenvironment. Acidosis is a key player in cancers, able to induce in tumor cells many aspects of malignancy, as invasiveness, resistance to apoptosis and drugs.

METHODS

We used male (SSM2c) and female (M51) primary cultures isolated from clinical comparable cutaneous metastatic melanoma. Melanoma cells were treated for 24 hours with acidic medium at pH 6.7.

RESULTS

We found that low pH induces an increased expression of ERß in M51 cells, while it causes a reduction of ERß in SSM2c. Moreover, acidosis promotes the expression of several EMT markers in SSM2c cells, with a reduction of IkB, while M51 cells kept EMT markers unchanged. In order to verify the role of ERß in EMT of acidic melanoma cells, we evaluated EMT markers after silencing the protein-coding gene of ERß (ESR2) and we found that in both ESR2 silenced cell lines, EMT markers' expression was up-regulated. To confirm this result, we decided to isolate, by sorter technique, sub-populations of melanoma cells at high and low ERß expression: the analysis indicated that levels of EMT markers were inversely related to ERß expression.

CONCLUSIONS

Overall, this study discloses a gender-dependent ERß expression in a well known pro-tumoral microenvironmental contest driving EMT. This finding may lead to the development of new gender personalized strategies for the treatment of melanoma.

P014

COMBINING CARBONIC ANHYDRASE INHIBITORS AND COBALT-BASED CARBON MONOXIDE RELEASING MOLECULES: DESIGN AND COUNTERACTION OF INFLAMMATION-RELATED DISEASES

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BACKGROUND-AIM

Carbon monoxide (CO) has been reported as an anti-inflammatory and cytoprotective substance at low concentrations. However, the administration of CO is complicated due to its gaseous state and obtaining specific cell responses is challenging. Thus, CO releasing molecules (CORMs) have attracted interest to downregulate immune and inflammatory responses in both in vitro and in vivo models such as Rheumatoid Arthritis (RA), Rotator Cuff Tears and Disease. Moreover, it has been reported that Carbonic Anhydrase (CA) is involved in the pathogenesis and maintenance of inflammation-related diseases. Levels of the isoform IX have been found in human fetal tendons as a marker of mechanical stress and an overexpression of CA IX and XII in inflamed joints has been recently reported. In this light, the synthesis of small molecule hybrids consisting of CA inhibitors or not linked to a CORM section has been proposed.

METHODS

Evaluation of the CO released over time revealed the organic portion linked to the CORM section to influence the CO releasing properties. Conversely, the DCH insertion did not highly hamper the CA inhibition. In vivo pain relief efficacy studies in the RA rat model showed that some derivatives were more efficient in terms of intensity as well as time distribution when compared to the CAI and CORM administered separately, confirming the success of the hybridization strategy. These very promising results fostered our interest in studying the anti-inflammatory and anti-oxidant properties of such hybrids at a biological and molecular level on LPS-stimulated mouse macrophages and H_2O_2-stimulated tendon-derived human primary cells in comparison with N-acetyl cysteine and meloxicam, respectively.

RESULTS

The compounds counteracted the induced inflammation and some hybrids displayed a better profile in terms of enhanced viability, decreased cytotoxicity, and augmented cell proliferation in both the cell models. In the inflamed tendon cell model, compound 7, as a potent superoxide scavenger, exerted its action inhibiting the NF-κB translocation and downregulating iNOS, whereas compound 2 was more effective in increasing collagen I deposition.

CONCLUSIONS

Taken together, these data lay the grounds for further investigations for the use of CAI-CORMs in inflammatory-related diseases.

P015

IDENTIFICATION OF NEW GERMLINE ALTERATIONS INVOLVED IN FAMILIAL NON-BRCA BREAST CANCER SUSCEPTIBILITY BY RE-ANALYSIS OF EXOME SEQUENCING DATA

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BACKGROUND-AIM

Breast cancer (BC) is the most commonly diagnosed cancer in women worldwide. Inherited mutations in the high penetrance susceptibility genes BRCA1/2 account for about 20-25% of hereditary/familial BC cases, thus in-depth studies are needed to identify additional genes that may contribute to BC predisposition in the remaining part of familial BCs (75-80% of cases). Next-Generation Sequencing (NGS) technology provides a powerful method to accurately characterize the status of multiple genes simultaneously. Based on the continuous improvement of NGS technology, updated gene databases and tools for variants classification and interpretation, the re-analysis of whole genome/exome NGS data from BRCA1/2 negative (non-BRCA) familial BC cases may possibly reveal novel candidate predisposition variants and/or re-classify variants of uncertain significance (VUS).

METHODS

Publicly available exome sequencing data from 15 familial non-BRCA BC cases were re-analysed using a novel and original bioinformatic pipeline generated "in-house" as well as tools available online, in order to identify new genes/ variants potentially associated to BC susceptibility. Case-control analysis to evaluate BC risk estimate for mutation carriers was also performed.

RESULTS

The analysis revealed pathogenic/potentially pathogenic variants in new putative candidate BC susceptibility genes as well as VUS in genes already known to be involved in BC predisposition, not identified previously. In particular, among them, one case was carrier of the pathogenic variant c.298C> T (p.Arg100Trp) in RBBP8, a gene involved in DNA repair by homologous recombination that interacts with BRCA1; interestingly, the same variant has been recently identified in an early onset, high risk, non-BRCA BC patient. The variant c.5068_5071dupAACA (p.Ser1691fs) in DLEC1, gene involved in BC-associated pathway, was also identified in one BC patient. Interestingly, for this variant, a significant association with high risk of BC emerged by case-control analysis.

CONCLUSIONS

Overall, these results highlight that the improvement of bioinformatic tools and the re-analysis of NGS data can lead to the identification of variants of potential clinical relevance in BC, not highlighted previously.

P016

OLEOCANTHAL TO OVERCOME GASTRIC CANCER RESISTANCE: A POSSIBLE NEW COMPLEMENTARY THERAPY

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BACKGROUND-AIM

Gastric Cancer (GC) is one of the most critical problem for Health System with more than one million new cases per year being the sixth malignancy for incidence and the fourth for mortality globally.
The only curative approach remains surgery, but chemotherapy can be used in combination or when surgery is not possible. However, also chemotherapy failed especially when patients undergo resistance, and this is particularly frequent.
In oncology, the use of extra-virgin olive oil (EVOO) has gained the interest of many scientists thanks to its multiple biological activities and its extremely low toxicity for the organism. The possibility to use nutraceutics in association with chemotherapeutic agents to enhance effectiveness of treatment and eventually reduce their dose to limit side effects, could represent a breakthrough in the tumor therapy. In particular, a compound of EVOO, Oleocanthal (OC), which is characterized by an ibuprofen-like chemical structure, shows effects in many types of cancer. The aim of this work is to verify whether OC might be useful to overcome GC resistance to therapy.

METHODS

We used the AGS gastric adenocarcinoma cell line and the AGS-resistant cell subpopulations selected, in our laboratory, trough chronic exposure of 5-fluorouracil (AGS-5FUr), cisplatin (AGS-CISr) or paclitaxel (AGS-TAXr). Cells exposed to different doses of OC were analyzed through MTT, AnnexinV-PI cytofluorimetric assay and cell cloning ability.

RESULTS

We disclose that 60 μM OC promotes the death of wild type AGS, AGS-5Fur and AGS-TAXr, but not AGS-CISr. We suggest that OC efficacy may be due to cell cycle inhibition in accordance with its ability to modulate p21 expression, as Western Blot analyses revealed. Is still obscure the AGS-CISr-OC resistance.

CONCLUSIONS

These preliminary results open-up the possibility to evaluate efficacy of OC in vivo, as valuable adjuvant in GC chemotherapeutic treatment, able to reduce chemotherapy resistance.

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P017

BCLAF1 FUNCTIONALIZATION AGAINST RELAPSED/REFRACTORY NEUROBLASTOMA

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BACKGROUND-AIM

Neuroblastoma (NB) is an extra-cranial solid tumor, arises from neural crest cells of sympathetic nervous system (SNS). Up to 75% of NB arise in abdomen leading to the worst prognosis of all the possible sites for the disease: 60% of the patients have metastases at diagnosis and the survival rate is <40%. Actually there isn't an effective therapy against recurrent NB, the survival rate for these patients is <5%[1,2,3]. NB shows features common to Acute Myeloid Leukimia (AML), the most important is related to differentiation defects and tumorigenesis: the balance miR-194-5p/BCLAF1 regulates differentiation and survival of normal hematopoietic progenitors and when is perturbed cells are locked into an immature state. SAHA treatment increase miR-194-5p expression leading to differentiation and apoptosis by inducing BCLAF1 shuttle between nucleus (pro-apoptotic role) and cytosol (differentiation role)[4]. The project aim is characterize BCLAF1 as new target for prognosis, diagnosis and therapy in NB.

METHODS

Cellular and molecular studies: PCR, Real-Time PCR, Western blot; in vivo analysis: immunofluorescence (IF) in mouse model; in silico analysis: RNA-seq data of primary NB samples.

RESULTS

Our preliminary data suggest a correlation between BCLAF1 expression level, NB development and drug-resistance. Using engineered metastatic NB cells selectively resistant to different conventional chemotherapy it was verified that BCLAF1 is over-expressed in metastatic cells and responsible of cisplatin-resistance: BCLAF1 is higher in cluster suspension subpopulation cells which are responsible for metastasis. In addiction BCLAF1 was identify in progenitor cells of SNS in early sympathetic ganglia by in vivo IF. BCLAF1's expression seems to be mainly cytoplasmatic, this localization could be linked to its role in differentiation commitment. SAHA (HDACi) appears to modulate BCLAF1's mRNA and protein levels also in SHSY5Y and UKF-NB4 cells. Finally, SAHA leads to decrease of BCLAF1 full-length isoform while seems increase exon5 truncated isoform, oncogenic and anti-cancer respectively. Functional study of these two isoforms is in progress.

CONCLUSIONS

These promising data suggest further investigations for deeper BCLAF1 molecular and genetic study in NB tumor.

P018

POTENTIAL ROLE OF PHOSPHORYLATED TAU IN FACILITATING CELL CYCLE PROGRESSION IN PROSTATE CANCER CELLS

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BACKGROUND-AIM

Tau protein is expressed in several human tumors including prostate, breast, stomach, pancreas and rectal colon cancer where it shows structure and functions similar to those described in neuronal tissue. Tau is mainly involved in the assembly and stabilization of microtubules, playing a role not only in the axonal signal transmission but also in the regulation of mitosis. As demonstrated in neurodegenerative diseases classified as tauopathies, etiology is associated with hyper-phosphorylation of the Tau protein that detaches from microtubules generating Tau oligomers and inducing cell cycle re-entry. One of the hallmarks of cancer is the loss of checkpoints during mitosis, and chemotherapeutic drugs currently in use such as taxanes aim to induce apoptotic death in the G2/M phase of the cell cycle as a result of interference in mitotic spindle assembly.

METHODS

We used DU145, PC3 and LNCAP cell lines as a reference models for prostate cancer. In these cells we investigated in vitro the status of Tau protein in mitosis and the effect of its modulation on M-phase progression also in combination with antimitotic drugs.

RESULTS

Tau protein was basally expressed in prostate cancer cell lines as several monomeric low-phosphorylated forms, with more aggressive cell lines expressing the higher levels of the protein. Immunofluorescence analysis revealed that Tau was visible mainly in dividing cells where it was localized on the mitotic spindle. Tau silencing was associated with a synergic antiproliferative effect with taxanes mainly evident in Tau high-expressing cell lines. The treatment with paclitaxel determined an accumulation of hyper-phosphorylated Tau forms that was reversed by treatment with CDK5 inhibitor, roscovitine. In parallel, the inhibition of Tau phosphorylation determined a reduction in the capacity to re-enter in cell cycle after M phase block.

CONCLUSIONS

Our data indicate that Tau phosphorylation is an important event in the modulation of cell cycle progression in tumor cells. The inhibition of tau phosphorylation could exert an anti-tumoral effect during chemotherapy counteracting the resistance mechanism associated with failure of G2/M checkpoints. Thus, therapeutic strategies aimed at opposing Tau protein homeostasis, could be an effective adjuvant in cancer therapy.

P019

GENE DOSAGE-DEPENDENT EFFECT OF NBS1 INACTIVATION ON SHH-DRIVEN MEDULLOBLASTOMA DEVELOPMENT.

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BACKGROUND-AIM

Nbs1 is essential for the DNA damage response (DDR) and its hypomorphic mutations cause the DDR-defective Nijmegen Breakage Syndrome, characterized by neurodevelopmental problems, immune defects and cancer predisposition. In mice, CNS-restricted Nbs1 KO leads to microcephaly and cerebellar ipoplasia. Extensive work from several labs including ours indicates NBS1 is essential to control the deleterious effects of replication stress (RS) and DNA damage, like those induced by MYCN during SHH-driven developmental expansion of cerebellar granule cell precursors (GCPs).
Coherently, Nbs1 is overexpressed in several tumors and the activity of the MRN complex, to which it belongs, is indispensable to restrain RS in MYCN-driven tumors, suggesting NBS1 (and by extension the MRN complex) might be essential in these tumors.
Mutations in the Nbs1 gene are linked to higher incidence of medulloblastoma (MB), an embryonal tumor of the cerebellum, in agreement with its well-established oncosuppressor role.Here we clarified which role Nbs1 has in MB development taking advantage of a new animal model.

METHODS

We generated and analyzed mono- or biallelic deletion of the Nbs1 gene in the context of the SmoA1 transgenic mouse, a SHH-MB prone animal model. In vitro manipulation of primary GCPs from WT and genetically modified animals, as well as Nbs1 knockdown via RNA interference in primary MB cells allowed biochemical analysis of the DDR, gene expression evaluation and examination of growth and clonogenic properties of these cells.

RESULTS

Nbs1 KO prevented MB development in SmoA1 mice and reduced tumor growth in a SHH-driven tumor allograft model. Ex vivo and in vitro analyses confirmed Nbs1 deficiency impairs SHH signalling.
In contrast, mono-allelic Nbs1 deletion increased tumor incidence in SmoA1 mice. While Nbs1+/- and WT MBs were almost indistinguishable by multiparametric analyses, Nbs1+/- GCPs showed not only a defective DDR but also increased clonogenic ability, at least in part assignable to enhanced Notch signalling.

CONCLUSIONS

Our study indicates that full Nbs1 KO is epistatic on SHH-driven MB development, but also that Nbs1 is haplonsufficient for SHH-MB development, thus revealing a gene dosage-dependent effect of NBS1 inactivation on SHH-MB development.

P020

ANALYSIS OF MOLECULAR TCGA DATA BY USING FEATURE EXTRACTION AND MACHINE LEARNING APPROACHES AIMED AT DISTINGUISHING TUMOR TYPES.

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BACKGROUND-AIM

Cancer is a complex disease, with patterns of genomic alterations showing intra-cancer heterogeneity and cross-cancer similarity. Molecular data spanning most cancer types can shed light on tumor molecular mechanisms and refine prevention, diagnosis and treatment in the personalized medicine era. Machine learning (ML) represents a powerful tool to capture relationships between molecular alterations and cancer types and build accurate methods to extract biological information. Starting from TCGA data, we developed a ML model aimed at distinguishing cancer types with high accuracy based on molecular lesions. This could improve cancer diagnosis by using specific DNA alterations, embedded in a replicable easy-to-use automated technology.

METHODS

Data of 9927 samples spanning 32 different cancer types were downloaded from cBioportal. To create datasets, calls for somatic point mutations (SNP, DEL, INS and ONP) and copy number alterations (CNV) at chromosome arm-level were considered as predictive features of cancer types. Preprocessing and XGBoost classifier models were applied. Due to imbalance in the dataset ascribable to different number of cases for each tumor, 4 differentially arranged homogeneous groups of datasets were created, based on the relative numerosity of cancers, and a XGBoost was built on each of them.

RESULTS

The 4 analysis models achieved 80%, 81%, 80%, and 76% accuracy, with AUC scores above 90%. Dimensionality reduction through feature selection in terms of importance/ranking produced models with similar accuracy/precision. Another global dataset was created to build a XGBoost model capable to link each group to a specific cancer type and used to technically confirm the appropriateness of the model, reaching an accuracy of 68% on the holdout data and a 84% AUC score. Overall, accuracy was improved by joining CNV with point mutations.

CONCLUSIONS

A new accurate ML model discriminating among 32 different cancer types was developed. This approach could have potential clinical application, showing how chromosome and gene mutation datasets can be used to distinguish tumor types. Further analyses to confirm the performance of this promising model, with more validation datasets, are required to extract information of biological relevance.

P021

NOTCH1 PROTEIN ANALYSIS IN HAILEY - HAILEY DISEASE

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BACKGROUND-AIM

Hailey-Hailey disease (HHD) is a rare autosomal dominant genetic disorder, caused by mutations in the ATP2C1 gene. At the cellular level, its deregulated function causes desmosomal alteration and loss of cohesion between keratinocytes, resulting in suprabasal acantholysis. Loss of function of ATP2C1 determines the increase of oxidative stress and the deregulation of the NOTCH1 signaling pathway. The aim of this project is to investigate the role of NOTCH1 protein in HHD disorder.

METHODS

We used a model system consisting of a stabilized line of keratinocytes (HaCaT) silenced for the gene responsible for the disease by RNA interference and transfection with liposomal vectors (si-ATP2C1), in order to investigate the crosstalk between ATP2C1 and Notch1 in the pathogenesis of HHD. Moreover, we used immunoblots with nucleus/ cytosol fractionations and immunofluorescent assay for our studies.

RESULTS

Through immunoblots with nucleus/cytosol fractionations and immunofluorescent assay we showed the cytosolic localization of NOTCH1 and its colocalization with LAMP1, an endosomal complex protein. We observed that inactivation of ADAM10 and ADAM17 proteins (si-ADAM10, si-ADAM17), required for the proteolytic activation of NOTCH1 signaling, did not affected siATP2C1-induced NOTCH1 cleavage, suggesting a non-canonical activation of NOTCH signaling. However, despite the presence of activated NOTCH1 protein we found that the expression of a subset of NOTCH1 target genes remain repressed in ATP2C1-defective keratinocytes. We provide evidence that in ATP2C1-defective cells NOTCH1 is targeted to the lysosomal compartment. Chloroquine treatment (a lysosomal inhibitor) on si-ATP2C1 treated cells stabilizes and promotes the nuclear translocation of the cleaved NOTCH1 protein (N1ICD).

CONCLUSIONS

Previous results in our research group revealed a possible involvement of NOTCH1 in Hailey - Hailey disease. In the present work, we show that loss of ATP2C1 function negatively regulates protein levels of active NOTCH1 in a post-translational manner that requires lysosomal activity.
An interesting model emerging from our experiments is that Notch might serve through a non-canonical signaling to titrate pathway levels that control keratinocyte proliferation/differentiation and in turn Hailey-Hailey disease manifestation.

P022

HEAD-TO-HEAD COMPARISON OF PHI AND PROCLARIX FOR THE IDENTIFICATION OF CLINICALLY SIGNIFICANT PROSTATE CANCER

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BACKGROUND-AIM

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and the second leading cause of male cancer related deaths. Widespread use of PSA led to a high rate of overdiagnosis and overtreatment. New diagnostic tools are needed to identify clinically significant PCa (csPCa) and choose a personalized treatment. In the recent years, several PCa biomarkers have been proposed with a clear tendency towards the use of panels of biomarkers or a combination of biomarkers and clinical variables. Among them, there were Prostate Health Index (PHI), based on a mathematical combination of the molecular forms of PSA, and the most recent Proclarix, an index score based on the evaluation of two glycoproteins (thrombospondin-1 and catepsin D) total and free PSA and the age of the patient. The aim of our study was to perform a head-to-head comparison of Proclarix and PHI.

METHODS

Before prostate biopsy (minimum 16 cores), 345 subjects were enrolled, and blood specimens were collected. Whole blood was allowed to clot before serum was separated by centrifugation. Serum aliquots were stored at −80 °C until samples were processed. Specimens were analyzed in blinded fashion for PSA, fPSA and p2PSA by Access2 Immunoassay System analyzer (Beckman Coulter, Brea, CA, USA) calibrated against the WHO standard for PSA and fPSA. Thrombospondin-1 and catepsin D were measured using the CE-marked Proclarix kit (Proteomedix). Comparison of AUC and performance at predefined cut-offs was performed to predict csPCa. Decision curve analysis was used to compare clinical benefits.

RESULTS

Roc curve analysis showed that Proclarix and PHI had a similar performance for predicting csPCa (AUC 0.78 vs 0.74, p=0.369). However, PHI had higher specificity at the cut-off of 40. Decision curve analysis showed that the combination of Proclarix and PHI had the highest clinical net benefit.

CONCLUSIONS

In this study, Proclarix and PHI showed similar performance in the prediction of csPCa. The combination of the two tests had the best performance avoiding 50% of unneeded biopsies, missing a very low percentage of csPCa.

P023

MOVING FORWARD TETANUS PROPHYLAXIS AND TREATMENT: TWO EXCEPTIONALLY POTENT HUMABS ARE EFFECTIVE IN MOUSE MODELS AND READY FOR CLINICAL TRIALS

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BACKGROUND-AIM

Tetanus neurotoxin (TeNT) is the causative agent of tetanus, a life-threatening disease of vertebrates, including humans, characterized by neurogenic muscle rigidity and spasticity.
Although tetanus can be prevented by a very effective vaccine, a worldwide clinical practice in the emergency rooms is the administration of anti-TeNT immunoglobulins (TIG), which are used both for prophylaxis to avoid tetanus development in wounded patients or for therapy to treat patients already carrying tetanus symptoms. TIG is produced from the blood of hyperimmune individuals, either humans or horses (in developing countries). As such, it exposes patients to several possible side-effects, including infections by still-unknown pathogens as well as dangerous anaphylactic reactions. Human monoclonal antibodies (humAbs), which are emerging as superior therapeutics against several diseases, have the potential to overcome the drawbacks of TIG

METHODS

By screening immortalized memory B cells pooled from the blood of immunized human donors, we isolated two humAbs, dubbed TT104 and TT110, which display an unprecedented neutralization ability against TeNT. We produced the Fab derivatives of TT104 and TT110 and determined the epitopes they recognize via cryo-EM. We also performed a battery of biochemistry, imaging and cell biology experiments to defined the molecular basis explaining how TT104 and TT110 interfere with TeNT mechanism of neuron intoxication.

RESULTS

TT104 and TT110 bind two epitopes required for TeNT binding to target neurons and entry inside the neuronal cytosol, respectively. Our analyses unraveled the molecular bases explaining the exceptional neutralization ability of TT104 and TT110 and, at the same time, shed novel light onto these two steps essential to TeNT activity in neurons.
Crucially, the combination of TT104 and TT110 display a prophylactic activity in mice when injected long before TeNT and the two Fab derivatives (TT104-Fab and TT110-Fab) neutralize TeNT in post-exposure experiments. Of note, in both these two paradigms of experimental tetanus, the humAbs and the Fabs show an activity fully comparable to TIG.

CONCLUSIONS

TT104 and TT110 humAbs and their Fab derivatives meet all requirements for being considered for prophylaxis and therapy of human tetanus and are ready for clinical trials.

P024

EMT AND DYSREGULATED AUTOPHAGY IN PANCREATIC DUCTAL ADENOCARCINOMA: INVOLVEMENT OF FGFR2 ALTERED SPLICING AND OF PKCε DOWNSTREAM SIGNALING.

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BACKGROUND-AIM

Pancreatic ductal adenocarcinoma (PDAC) is a treatment-resistant malignancy characterized by a high malignant phenotype including acquired EMT signature and deregulated autophagy. Since we have previously described that the aberrant expression of the mesenchymal FGFR2c isoform and the triggering of the downstream PKCε signaling are involved in epidermal carcinogenesis, aim of this work has been to assess the contribution of these oncogenic events in PDAC progression.

METHODS

Biochemical, molecular and immunofluorescence analysis combined with the use of FGFR2 inhibitor or specific RNA silencing approaches by shRNA, were performed on Panc-1 and Mia PaCa-2 cell lines, expressing different levels of FGFR2c, to evaluate FGFR2c downstream signaling pathways, EMT profile and the modulation of autophagy in response to FGF2.

RESULTS

We found that FGFR2c expression impacts on PDAC cell responsiveness to FGF2 in terms of intracellular signaling activation, upregulation of EMT-related transcription factors and modulation of epithelial and mesenchymal markers compatible with the pathological EMT. Moreover shut-off via specific protein depletion of PKCε signaling, activated by high expression of FGFR2c resulted in a reversion of EMT profile, as well as in a recovery of the autophagic process. The detailed biochemical analysis of the intracellular signaling indicated that PKCε, bypassing AKT and directly converging on ERK1/2.

CONCLUSIONS

Overall, our results indicate that PKCε could be a crucial hub signaling molecule downstream FGFR2c and represents an effective therapeutic target in counteracting PDAC aggressive phenotype.

P025

NF-KB/CES1 AXIS PROMOTES METABOLIC ADAPTATION IN AGGRESSIVE COLORECTAL CARCINOMA

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BACKGROUND-AIM

The ability to adapt to low-nutrient microenvironments is essential for tumor-cell survival and progression in solid cancers, such as colorectal carcinoma (CRC). Signaling by the NF-κB transcription-factor pathway associates with advanced disease stages and shorter survival in CRC patients. NF-κB has been shown to drive tumor-promoting inflammation, cancer-cell survival and intestinal epithelial cell (IEC) dedifferentiation in mouse models of CRC. However, whether NF-κB affects the metabolic adaptations that fuel aggressive disease in CRC patients is unknown.

METHODS

We interrogated the consensus molecular subtype (CMS) classification of CRC as a starting point to investigate the impact of NF-kB in driving metabolic adaptation of cancer cells.

RESULTS

We identified carboxylesterase 1 (CES1) as an essential NF-κB-regulated lipase linking obesity-associated inflammation with fat metabolism and adaptation to energy stress in aggressive CRC. CES1 promoted CRC-cell survival via cell-autonomous mechanisms that fuel fatty-acid oxidation (FAO) and prevent the toxic build-up of triacylglycerols. We found that elevated CES1 expression correlated with worse outcomes in overweight CRC patients. Accordingly, NF-κB drove CES1 expression in CRC consensus molecular subtype (CMS)4, associated with obesity, stemness and inflammation. CES1 was also upregulated by gene amplifications of its transcriptional regulator, HNF4A, in CMS2 tumors.

CONCLUSIONS

Our findings underscore the clinical relevance of CES1 as a central driver of CRC downstream of NF-kB and suggest CES1 could provide an effective route to treat CRC patients with particularly poor prognosis.

P026

HYPERVAIBLE-LOCUS MELTING TYPING (HLMT): A NOVEL, FAST AND INEXPENSIVE APPROACH TO PATHOGEN TYPING. APPLICATION IN A YEAR-LONG REAL TIME NOSOCOMIAL SURVEILLANCE PROGRAM

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BACKGROUND-AIM

Healthcare-associated infections are a major burden for public health world-wide. Hospital surveillance is one of the most effective strategies to control pathogen spreading in hospital settings. Multi Locus Sequence Typing (MLST) and Whole Genome Sequencing (WGS) are common methods for pathogens typing in hospitals. These methods are expensive and/or time consuming and/or require specialized skills. This has limited their application in hospital real time surveillance programs. High Resolution Melting (HRM) is a PCR based method to discriminate amplicons on the basis of their melting temperatures and it has the potential to be used for pathogen typing. Despite this, HRM application in hospital settings is limited because it is challenging to develop novel discriminatory typing protocols and to interpret data.

METHODS

Here we present Hypervaible-Locus Melting Typing (HLMT), a novel approach to easily develop highly-discriminatory HRM protocols that can be applied to perform fast, repeatable pathogen typing and epidemiological investigations. We already published and used the software EasyPrimer to develop an HRM protocol for Klebsiella pneumoniae typing. Then, we developed and used the software MeltingPlot to type all the K. pneumoniae isolates (n=80) collected in 2017 in a large hospital in Milan (Italy). Lastly, we compared HLMT results with in-silico MLST and WGS.

RESULTS

Hypervariable-Locus Melting Typing is able to provide an accurate description of the epidemiological scenario: the epidemiological curves obtained using HLMT data are comparable to the curves built using MLST and WGS for the two most prevalent clones. Even if HLMT is less precise than WGS, it is able to correctly identify the two most prevalent clones against the sporadic clones isolated in the hospital.

CONCLUSIONS

Considering that Hypervariable-Locus Melting Typing (HLMT) is faster (~5 hours) and less expensive (~5 euros per isolate) than MLST and WGS (~50 and ~100 euro per isolate in a few days), this result clearly shows that HLMT is suitable for real time surveillance programs in hospital settings.

P027

CANDIDATE GENE IDENTIFICATION IN BRUGADA SYNDROME BY NEXT GENERATION SEQUENCING

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BACKGROUND-AIM

Brugada Syndrome (BS) is an inherited disease characterized by risk of sudden cardiac death in young asymptomatic adults. So far, 22 genes have been associated with BS susceptibility but 70% of patients still remain genetically undiagnosed. To identify new candidate genes underlying BS phenotype, we exploited whole-exome sequencing (WES) approach in a total of 124 Brugada patients, including 102 sporadic patients, of which 81 were negative for SCN5A mutation, and 22 genetically undiagnosed familiar cases.

METHODS

Genomic DNA was enriched for exome libraries (Agilent SureSelect QXT v.7 kit and XT HS kit) and sequenced on NextSeq500 and NovaSeq S2 6000 Illumina. Raw reads were analyzed using an in house pipeline and Dragen Bio IT Platform. Prioritization analysis was performed focusing on rare variants (MAF ≤0.05%), localized within coding sequence or in splice-site regions, segregating in affected patients. Candidate variants were classified according to ACMG guidelines, with the support of eVai-EnGenome software.

RESULTS

WES obtained a mean coverage of 180X and 97% of target region had a read depth >20X. We detected about 200 rare variants per sample. Subsequent prioritization analysis highlighted 2-3 candidate variants per case. After ACMG classification, we focused only on pathogenic, probably pathogenic and unknown significance variants. The selected variants are private and most of them are missense (90%). They are localized in genes mainly encoding for ion channels, regulatory proteins of cardiac excitability, sarcomeric protein, nuclear and junctional proteins, suggesting their possible role in BS phenotype.

CONCLUSIONS

Despite BrS has always been considered as a channelopathy, our results underline the emerging role of structural genes in the clinical phenotype manifestation, even if further studies are necessary to confirm their pathogenic role. We are now going to perform a Burden Testing approach, allowing to bring out the most enriched mutated genes, supporting their possible pathogenic role. Moreover, we are setting up a pipeline for copy number variation (CNV) analysis. In perspective, we aim at investigating genotype-phenotype correlations, based on the available genetic and clinical database, to ameliorate risk stratification criteria.

P028

MECHANISTIC INSIGHT INTO ESTROGEN RECEPTOR VARIANT ERα46 COOPERATION WITH INSULIN RECEPTOR TOWARD GROWTH AND METASTASIS OF PRIMARY BREAST CANCER CELLS

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BACKGROUND-AIM

The estrogen receptor (ER)α splice variant ERα46 has been implicated in estrogen responses in addition to ERα66kDa full-length. However, the mechanisms regulating ERα46 expression and function remain unknown. Together with estrogen signals, the insulin/insulin receptor (IR) axis has been demonstrated to contribute to breast cancer (BC) progression. In this regard, estrogen and insulin signaling have been shown to orchestrate a cross-communication that facilitates aggressive and life-threatening BC phenotypes.

METHODS

A novel naturally immortalized BC cell line, named BCAHC-1, was isolated from a patient with invasive ductal BC. To provide further mechanistic insight into the molecular features triggering BC development, we performed in BCAHC-1 cells RNA-seq analysis, reporter gene, gene expression and silencing experiments, immunoblot and co-immunoprecipitation, immunofluorescence and electron microscopic studies, ELISA, flow cytometry, proliferation, clonogenic and spheroid formation assays, xenograft mouse models of tumor growth and lung metastases.

RESULTS

BCAHC-1 cells showed a unique expression of IR along with ERα46, as assessed using both ERα C-terminal and N-terminal antibodies. A functional cooperation between these receptors was found in BCAHC-1 cells upon estrogen and insulin stimulation toward cancer growth and pulmonary metastasis. Transcriptome profiling of BCAHC-1 cells identified interleukin-11 (IL11) as a main factor by which BCAHC-1 cells trigger invasive and migratory effects in cancer-associated fibroblasts (CAFs). Finally, bioinformatics analysis revealed the association of IL11 with poor outcomes and pro-invasive pathways in BC patients.

CONCLUSIONS

The peculiar receptor expression profile of BCAHC-1 cells provides a valuable tool to determine novel molecular mechanisms involved in the progression of certain BC subtypes. Moreover, these findings may pave the way for the usefulness of targeting ERα46 and IR in novel therapeutic options in BC patients characterized by dysregulated estrogen and insulin signaling pathways. Of note, the translational meaning of our data may also suggest the usefulness of antibodies recognizing either C-terminal or N-terminal domains of ERα in order to assess its expression as full-length and/or splicing variants toward more appropriate therapeutic settings.

P029

COMPUTATIONAL ANALYSIS UNRAVELS GPER-ASSOCIATED NETWORK PREDICTING ADVERSE FEATURES IN ERα - NEGATIVE BREAST CANCER

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BACKGROUND-AIM

The multifaceted biological effects exerted by estrogens are mainly mediated by estrogen receptor (ER)α and ERβ. In addition, the G-protein-coupled estrogen receptor (GPER) has been involved in the rapid responses to estrogens leading to breast cancer (BC) progression. Accordingly, GPER over-expression has been correlated with lower survival rates, increased tumor size and higher risk of developing metastatic disease in BC patients. Therefore, we performed a bioinformatics analysis on ERα-negative BC datasets to uncover the GPER-associated gene expression landscape for a better assessment of its action in tumor development.

METHODS

Computational studies were performed with R Studio using mRNA expression data and clinical information of the TCGA and METABRIC datasets. The cor.test() and the intersect() functions allowed us to obtain the most positively GPER-correlated genes shared by the abovementioned datasets. Pathway enrichment analysis was carried out using the ReactomePA package, whereas Gene Set Enrichment Analysis was performed with the phenoTest package. The survival analyses were accomplished using the gene expression data of the TCGA patients along with the disease-free interval information, employing the survminer and survivALL packages.

RESULTS

In ERα-negative BC patients we found that the levels of GPER are positively correlated with genes involved in pro-invasive processes. Performing pathway enrichment analysis, we assessed that 260 GPER-correlated genes shared by the two datasets are mainly enriched in pro-migratory and metastatic signaling pathways belonging to the extracellular matrix rearrangement. Further corroborating these findings, we ascertained that the genes included in the aforementioned transduction pathways are highly expressed in ERα-negative tumors with a most abundant stromal component. Then, elevated expression levels of both GPER and certain genes of the aforementioned metastatic pathways were found predictive of a poor outcome in ERα-negative BC patients.

CONCLUSIONS

Our data provide novel insight regarding the role of GPER-associated network in ERα-negative BC still needing more comprehensive therapeutic approaches.

P030

THE NOVEL ROLE OF PKG IN THE PHOSPHORYLATION AND AGGREGATION PROCESSES OF TAU PROTEIN

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BACKGROUND-AIM

Tau protein can be phosphorylated by multiple kinases at several sites. Among such kinases, the cAMP-dependent protein kinase A (PKA) phosphorylates tau at Ser214. This event exerts a protective effect against the assembly of tau into aggregated structures that are associated to the pathogenesis of Alzheimer's disease and other neurodegenerative disorders. The activation of PKA by cAMP also sustains long-term potentiation (LTP), a form of synaptic plasticity, through the stimulation of Aß production. In a similar manner, cGMP was found to boost Aß levels and to favor LTP and memory, but an effect of cGMP on tau phosphorylation has never been reported. To investigate this issue, we evaluated the possibility of a PKG-induced phosphorylation of tau.

METHODS

The phosphodiesterase 5 inhibitor vardenafil was used to increase cGMP levels in different model systems: neuro N2a cells, rat hippocampal slices and adult male C57BL/6 mice. Phosphorylation of tau was analyzed by immunoblotting, gene silencing, in vitro enzymatic assays and nano-HPLC mass spectrometry. Aggregation of tau was evaluated by gel electrophoresis.

RESULTS

Our data show for the first time that the cGMP-activated PKG phosphorylates tau at Ser214. Additionally, other 7 Ser/Thr tau residues appear to be phosphorylated by this kinase, but not Ser202, which is considered one of the earliest markers of tau aggregation. Furthermore, preliminary results indicate that PKG phosphorylation could reduce the aggregating capacity of tau.

CONCLUSIONS

Our results demonstrate the existence of a PKG-mediated mechanism that might shift tau from a pro-aggregant to an anti-aggregant conformation, which has been reported to exert neuroprotective functions.

P031

ANTIPROLIFERATIVE STRATEGY TO IMPROVE THE OUTCOME OF GLAUCOMA SURGERY

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BACKGROUND-AIM

Elevated intraocular pressure (IOP) is a major risk factor for glaucoma and the only target of current therapies. In eyes not responding to IOP-lowering drugs, IOP can be reduced by surgery. However, conjunctival and subconjunctival scarring limits the long-term success of several glaucoma surgical procedures. Anti-metabolites treatment during surgery or follow-up can help physicians to keep the surgery functioning, but with some ocular side-effects.
This study aimed to identify a drug that inhibits the proliferation of the Tenon's capsule fibroblasts that cause episcleral fibrosis, potentially improving the surgical outcome.

METHODS

Primary human Tenon's fibroblasts (HTFs) were isolated from tissue explants and characterized by immunofluorescence. The cytotoxic potential of the tested drugs was analysed with the MTT test, while the migration and proliferation of HTFs were assessed by the wound-healing assay.

RESULTS

Taken all together, the results obtained indicate that: 1) at least in our experimental conditions, HTFs proliferation, rather than migration, is responsible for wound healing; and 2) among the antiproliferative drugs tested, pemigatinib and sorafenib significantly slow the rate of wound closure.

CONCLUSIONS

Pemigatinib and sorafenib, two drugs already approved for cancer treatment, could represent a new therapeutic approach for improving the outcome of glaucoma surgery. Future evaluations in animal models will help determine their actual efficacy and safety in vivo.

P032

CYCLOSPORINE A INHIBITS SARS-COV-2 VIRAL INFECTION AND RELEASE, AS WELL AS CYTOKINE PRODUCTION IN LUNG CELLS

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BACKGROUND-AIM

Patients affected by COVID-19 are characterized by different clinical manifestations, which all have in common the hyper-activation of the immune system, even low-severity cases. Despite several approaches having been tested, no specific therapeutic protocol has been approved.
Cyclosporine A (CsA) interferes with viral infection and replication via sequestration of CyPA from binding to different viruses, i.e. VSV, HBV, HCV, HIV-1, and SARS-CoV. Moreover, CsA is known to be an immunosuppressor known to prevent T cell activation.
We investigated the effects of CsA on SARS-CoV-2 infection in a human pulmonary cell line; results showed that CsA hampers both viral infectivity and the production of proinflammatory cytokines by different variants of SARS-CoV-2, suggesting a potential exploitation of this drug in the therapy of COVID-19

METHODS

CaLu3 pulmonary cells were treated with 0.1, 1, or 10 μM CsA (together with Remdesivir as comparison) either before or after infection with SARS-CoV-2. The cytotoxic effect of CsA was monitored by MTT assay. Viral RNA was quantified at 48h by droplet digital PCR (ddPCR). Real-time PCR was employed to assess IL1a, IL6, IL8, TNFa, IFITM3 and CH25H mRNA expression. Protein concentration and localization was evaluated by western blot and immunofluorescence analysis. Finally, infectious virus particle was evaluated by TCID50.

RESULTS

Our findings show that: i) CsA-treated cells, either before or after SARS-CoV-2 infection, express significantly lower levels of Spike protein; ii) the RNA levels of nucleocapside were significantly decreased in cells treated with CsA treatment; iii) CsA treatment dampens the number of released infectious viral particles (evaluated by analyzing N1 RNA in the supernatant and by TCID50) in both experimental conditions; iv) CsA dampens the virus-induced synthesis of cytokines (i.e. IL6, IL8, IL1α and TNFα), type I IFN-modulated restriction factors (IFITM3) and cholesterol 25-hydroxylase. Similar results were obtained with all the different variants.

CONCLUSIONS

Altogether, these results suggest that CsA is able to counteract in vitro viral replication and to dampen the subsequent induction of cytokines in a human pulmonary cell line model. Therefore, CsA might be considered for repositioning to timely treat severe COVID-19 patients.

P033

ANTIBIOTICS TREATMENT IMPAIRS THE GROWTH OF MURINE TRIPLE NEGATIVE MAMMARY TUMORS BY ALTERING THE TUMOR ASSOCIATED MICROBIOTA.

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is the most aggressive BC subtype and a recent candidate for immunotherapy. Gut microbiota influences tumor growth and immunotherapy response. Other tissues host their own microbiota, that changing in tumor favours immune suppressive cells recruitment. In lung, we identified the targeting of local microbiota by antibiotic aerosol a strategy to promote an increased anti-tumor immunity.

METHODS

BALB/C mice were mammary fat pad transplanted with 5x104 4T1 TNBC cells and received aerosolized or oral Vancomycin /Neomycin, or Ampicillin, or Clindamicin, or saline starting 24 h later. The tumor immune microenvironment was analyzed by Real Time PCR and FACS; the tumor microbiome by 16S rRNA metagenomic analysis.

RESULTS

Aerosolized Vanco/Neo decreased spontaneous lung metastases in mice implanted with 4T1 cells (p=0,0193) and impaired mammary tumor growth (p<0,0001) by inducing an M1 local immunity. This effect depend on antibiotics systemic bioavailability, since oral Vanco/Neo, that has minimal access to the bloodstream at intestinal barrier, did not affect mammary tumor. In contrast, antibiotics that cross the intestinal epithelium, Amp or Clinda, impaired mammary tumor by aerosol and also orally (p<0,0001). CD3+ T cells and F4/80+ macrophages resulted expanded in tumors, suggesting that antibiotics affect mammary tumor growth by modulating the local microbiota and in turn the immune microenvironment. Accordingly, in untreated versus Amp-treated tumors we found increased level of pathogen-recognition receptors (PRRs) and a different representation of 8 bacterial taxa, as Staphylococcus genus, over-represented in untreated tumors, and Micrococcaceae genus, over-represented in Amp-treated tumors, by 16S rRNA metagenomic analysis. In vivo peritumoral transfer of Micrococcus luteus and Clostridium perfringens strains, that we isolated from Amp-treated tumors, significantly reduced the mammary tumor growth (p<0,0001 and p=0.0006), while no effect was induced by Staphilococcus epidermidis, isolated from untreated tumors.

CONCLUSIONS

Our findings support a role of the tumor associated microbiota on the growth of mammary tumor suggesting that its manipulation might create an immune microenvironement favourable to respond to immunotherapy in TNBC patients.

P034

PROTEIN-PROTEIN INTERACTION NETWORK ANALYSIS APPLIED TO DNA COPY NUMBER PROFILING SUGGESTS NEW PERSPECTIVES ON THE AETIOLOGY OF MAYER-ROKITANSKY-KüSTER-HAUSER SYNDROME: A ROLE FOR PRKX GENE

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BACKGROUND-AIM

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a rare disease, characterised by the agenesis of vagina and uterus in women with a normal 46,XX karyotype. Genetic alterations associated to MRKH syndrome have been detected only in a small percentage of patients. The aims of the present study are: to identify the presence of copy number variations (CNVs) in 36 MRKH patients; to depict a network of protein-coding genes located into the identified CNVs and to include them in MRKH-related pathways through in silico protein-protein interaction (PPI) analyses; to study the role of selected candidate genes in biological processes involved in the genital and urinary tract morphogenesis through in vitro analysis.

METHODS

DNA from peripheral blood samples from MRKH patients was analysed to assess the presence of CNVs by array-CGH and MLPA analyses. Quantitative RT-PCR experiments were conducted on RNA samples from vaginal vestibule tissue of MRKH patients. In vitro studies were performed on VK2 E6/E7 cell line.

RESULTS

Chromosomal aberrations were found in 9/36 patients. Microdeletions previously associated to MRKH syndrome, at 16p11.2 and 17q12 regions (encompassing TBX6, LHX1 and HNF1B genes), were detected in two cases; a third patient showed a duplication of CNE-2, a SHOX enhancer. One patient carried a heterozygous microduplication in Xp22.33, not yet described in MRKH patients, containing the PRKX gene, which encodes for a Ser/Thr cAMP-dependent protein kinase. A PPI network analysis highlighted the centrality of PRKX and that the most relevant biological connections are related to anatomical structure development. qRT-PCR demonstrated altered expression of PRKX in MRKH patients with respect to healthy controls. Using in vitro modelling, we proved that ectopic PRKX overexpression promotes cell motility and epithelial-to-mesenchymal transition activation in a vaginal keratinocytes cell line, two key processes in urogenital tract morphogenesis. Moreover, our findings showed that PRKX overexpression is capable of deregulating transcriptional levels of HOX genes implicated in urinary and genital tracts development.

CONCLUSIONS

We identified novel genetic alterations and interactions that may be likely involved in MRKH phenotype determination.

P035

DNMT3A AND DNMT3B TARGETING AS AN EFFECTIVE RADIOSENSITIZING STRATEGY IN EMBRYONAL RHABDOMYOSARCOMA

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BACKGROUND-AIM

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood. Despite a multimodal approach for RMS treatment, including surgery, chemotherapy and radiotherapy, the 5-year survival rates of high-risk RMS patients remain unfavourable. Recently, we demonstrated the overexpression of both DNA methyltransferase 3A (DNMT3A) and 3B (DNMT3B) in RMS tumour biopsies and cell lines compared to normal skeletal muscle. Radiotherapy may often fail due to the abnormal expression of some molecules able to drive resistance mechanisms. So, the aim of this study was to analyse the involvement of DNMT3A and DNMT3B in radioresistance-related mechanisms in RMS cancer.

METHODS

Before ionizing radiation (IR, 4 Gy) exposure, RNA interference experiments against DNMT3A and DNMT3B were performed in embryonal RMS cells. To assess the effects of the combined treatment on RMS cells, proliferation assay, flow cytometry analysis, Western blotting, q-PCR, immunofluorescence experiments and colony formation assays were performed.

RESULTS

Our findings demonstrate for the first time that the over expression of DNMT3A and DNMT3B levels may contribute to radiotherapy failure. Indeed, both DNMT3A and DNMT3B knocking down increased the sensitivity of RMS cells to IR as indicated by the drastic reduction of colony formation ability. Interestingly, DNMT enzymes act in two different ways: DNMT3A silencing triggers the cellular senescence program by up-regulating p16 and p21 cell cycle inhibitors, whilst DNMT3B depletion induces a significant DNA damage, as indicated by the strong activation of γ-H2AX marker and impairing the accumulation of key proteins of the DNA repair machinery (ATM, DNA-PKcs and Rad51). Moreover, our results demonstrate that the inhibition of p38 MAPK activity might contribute to radiotherapy resistance observed in RMS. Finally, the radiation treatment preserves the myogenic program triggered by DNMT3B silencing and induces irreversible DNA damage in undifferentiated cells.

CONCLUSIONS

We described for the first time the inhibition of DNMT3A and DNMT3B as a potential radiosensitizing strategy in the treatment of RMS. Our findings might contribute to improve the therapeutic protocols, mainly for patients with metastatic or recurrent RMS tumours.

P036

POTENTIAL ROLE OF CASPASE-1 IN THE REGULATION OF 5-LIPOXYGENASE EXPRESSION AND ACTIVITY: AN IN SILICO AND IN VITRO ANALYSIS IN SENESCENT ENDOTHELIAL CELLS

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BACKGROUND-AIM

When compared to their younger counterparts, senescent cells show a reduced proliferative potential, along with a pro-inflammatory phenotype, namely Senescence-Associated Secretory Phenotype (SASP). The SASP fuels the systemic chronic low grade inflammation observed in elderly individuals, universally named as inflamm-aging. 5-Lipoxygenase (5-LO) is the essential enzyme for the biosynthesis of leukotrienes (LTs), lipid mediators of inflammation whose overproduction contributes to several chronic inflammatory diseases. The double role of 5-LO in both promoting inflammation via LTs synthesis and resolving inflammation via lipoxins production raises the attention on the mechanisms of its modulation. Recent reports highlights 5-LO regulation by casp-6 and casp-8, which are known to mediate apoptosis. In this study, we investigated whether 5-LO could be modulated by pro-inflammatory caspases in an inflammatory environment, such as that of senescent cells.

METHODS

Senescent human umbilical vein endothelial cells (HUVEC) have been selected as cellular model and characterized. In silico techniques were launched to characterize the structure and the interaction of 5-LO and Casp-1, by means of computational bioinformatics tools and servers. Casp-1 activity in HUVEC was detected by FACS. Expression and cleavage of 5-LO was examined by western blotting and in vitro assay with Casp-1 recombinant protein.

RESULTS

We demonstrated an increased expression of 5-LO and an augmented activation of Casp-1 in senescent cells. An integrated in silico analysis, along with in vitro assays suggest that 5-LO could be a substrate of Casp-1 and that one of the most likely cleavage sites could be Asp176.

CONCLUSIONS

We identified 5-LO in HUVEC for the first time and showed an increased expression of the enzyme during senescence, along with an increased activity of Casp-1. We performed an in silico analysis which allowed us to detect a potential cleavage site in 5-LO protein sequence, in line with our experimental data. 5-LO turnover is extremely complex and the exact role of its modulation remains to be elucidated. Is Casp-1 cleavage a mere inactivation of the enzyme or is it a cellular attempt to control inflammation and promote resolution?

P037

TARGETING NOTCH3 RECEPTOR TO OVERCOME PLATINUM RESISTANCE IN OVARIAN CANCER

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BACKGROUND-AIM

Ovarian Cancer (OC) represents the fourth leading cause of cancer death in women. In this scenario, Platinum (PT)-based therapies, including the administration of Cisplatin and Carboplatin, represent part of the treatment regimen for OC-bearing patients. Unfortunately, PT-resistant tumor recurrences are frequently observed, thereby highlighting the need of novel therapeutic targets to restore PT-sensitivity. Notably, an increasing number of studies showed the crucial role played by Notch signaling in OC. Among the four Notch receptors, Notch3 (N3) is frequently over-expressed in OC and it associated to PT-resistance even if the role of N3 in this complex phenomenon is far from being fully understood. Nonetheless, since N3 is crucial for PT-resistance in OC, selective targeting of N3 may represent a promising therapeutic approach. Therefore, the main aims of this work are: 1. to dig deeper into N3-dependent PT-resistance; 2. to evaluate whether a previously identified positive regulator (PR) of N3 in T-cell acute leukemia (T-ALL) sustains N3 also in OC context.

METHODS

N3-positive and N3-negative established OC cell lines were used. We performed: pharmacological treatments, lentiviral transduction, in silico analysis on mRNA data collected by OC-bearing patients and immunohistochemistry (IHC) on OC tissue samples.

RESULTS

Firstly, we documented that PR sustains the expression of N3 intracellular domain in OC cell lines. Moreover, in silico analysis showed a significant direct correlation between PR and N3 gene expression levels in a cohort of OC-bearing patients and suggested that N3-PR crosstalk might be involved in the acquisition of aggressive phenotype. These observations were consistent with IHC performed on primary lesions from OC-bearing patients. Finally, in vitro studies documented the involvement of N3 in the promotion of PT-resistance which might be reversed by the pharmacological inhibition of PR, which results in defecting N3 signaling.

CONCLUSIONS

All in all, our findings give hints to further investigate the N3-PR relationship in OC: evaluating how PR inhibition affects N3-dependent OC behavior may aid in developing novel therapeutic strategies to restore chemo-sensitivity, finally improving the prognosis of OC-bearing patients.

P038

THE TRPM8 CALCIUM CHANNEL AS A NEW THERAPEUTIC TARGET IN PROSTATE CANCER.

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BACKGROUND-AIM

Prostate cancer (PC) is the most common malignancy in men. Identification of novel strategies that might avoid its progression and improve the standard clinical protocols is a primary need. The transient receptor potential melastatin-8 (TRPM8) represents an emerging molecular target implied in the migration and aggressiveness of PC cells. Its role in PC progression and drug resistance is, however, still pending. In our recent work, we have investigated the effect of new TRPM8 modulators in different biological responses of PC-derived cells. The molecular mechanism underlying their activity has been also disclosed.

METHODS

Patch-clamp and calcium fluorometric assays were used to characterize the synthesized compounds. Various PC-derived cell lines, at different degree of malignancy, were unstimulated or stimulated with androgens in the absence or presence of synthesized compounds. Cell proliferation and invasion were investigated in a preliminary screening. The most effective compounds were employed in PC 3D models. By using transcriptional and non-transcriptional reporter assays, we identified the intracellular molecular targets of our lead compounds. Biochemical approaches revealed the interference of selected compounds in the androgen-induced AR/TRPM8 complex assembly. At last, Ca2+ imaging experiments were done to assess the influence of TRPM8 modulators on the androgen-induced increase of intracellular calcium.

RESULTS

TRPM8 antagonists significantly inhibit the androgen-dependent PC cell proliferation and invasion. Moreover, the compounds remarkably affect the growth of PC cell spheroids. Analysis of pathways controlled by the androgen/androgen receptor (AR) axis shows that TRPM8 antagonists inhibit the non-genomic androgen actions and perturb the androgen-induced AR/TRPM8 complex assembly as well as the increase in intracellular calcium levels in PC cells. Notably, our findings show that the selected compounds are very effective in castrate-resistant PC cells or in PC cells expressing the ARV7 variant, which often confers resistance to the antiandrogen therapies currently used.

CONCLUSIONS

Our preclinical findings show that TRPM8 is a very promising target in PC. Its targeting by new modulators might be envisaged even when antiandrogen therapies fail.

P039

ADIPONECTIN MODULATES STEM CELL ACTIVITY IN HORMONE-RESISTANT BREAST CANCER

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BACKGROUND-AIM

Breast cancer (BC) is the most common cancer in women, both for incidence and mortality. In the current view, BC is considered a stem cell disease due to the presence of cancer cells with stem-like features and tumor-initiating potential. These cells, known as BC stem cells (BCSCs), can be recognized based on their ability to self-renew and the capacity to differentiate into the different cell types present in a tumor. It is increasingly clear that BCSCs possess high tolerability to chemotherapy, radiotherapy and hormone therapy, that make them responsible of disease relapse, tumor dissemination and metastasis.
The tumor microenvironment regulates stem cell proliferation and resistance to apoptosis through the secretion of cytokines and growth factors. In mammary microenvironment, adipocytes represent the most abundant cellular component and together with their secreted factors, they represent the main protagonists in the interactions between stromal and epithelial cells. Among the secreted factors, the adipokine adiponectin plays a key role in breast tumorigenesis.
Here, we investigated the effects of low adiponectin level, that commonly occurs in obese women, on BCSCs activity in hormone-resistant cells.

METHODS

We tested the ability of MCF-7 wild type (WT) and tamoxifen-resistant (TR) to grow as mammospheres (mammospheres forming efficacy, MFE), measuring the ability to maintain cell viability (self-renewal) upon serial non-adherent passages. RNA levels of stemness and EMT markers were evaluated by qRT-PCR. Mammosphere cell cycle was analyzed by flow cytometry.

RESULTS

Adiponectin treatment significantly enhanced MFE and self-renewal capacity in TR-MCF-7 cells compared to MCF-7 WT cells. qRT-PCR revealed that adiponectin increased the mRNA levels of stemness markers in TR-MCF-7 cells, grown as mammospheres. Interestingly, the flow cytometric analysis revealed a block of cell cycle in adiponectin-treated TR-MCF-7 mammospheres. The latter results together with decreased Ki67 expression and increased EMT markers may address tumor dormancy.

CONCLUSIONS

Our results demonstrated that low adiponectin level, as it occurs in obese BC microenvironment, driving EMT, enhances stem-like features in TR-MCF-7 cells to sustain tumor progression.

P040

HIGH LEVELS OF CIRCULATING MYELOID DERIVED SUPPRESSIVE-LIKE CELLS ARE ASSOCIATED WITH A LACK OF BENEFIT FROM IMMUNE CHECKPOINT INHIBITORS IN METASTATIC NON-SMALL CELL LUNG CANCER.

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BACKGROUND-AIM

Immune checkpoint inhibitors (ICIs) are part of the standard treatment for patients with metastatic non-small cell lung cancer (NSCLC). These drugs improved the tumor response and the survival outcomes in comparison with chemotherapy. However, some patients experience a primary resistance to immunotherapy. Currently, a defined marker has not been yet available to predict progressive disease (PD) at first imaging evaluation after the start of an ICI. Myeloid derived suppressive-like cells (MDSC) are already known to suppress the immunity against cancer cells via PD-L1 expression and TGF-beta release.

METHODS

Patients with metastatic NSCLC starting an ICI were included in this study. We drew a blood sample before starting the treatment. We performed a flow cytometric analysis of circulating monocytic MO-MDSC on peripheral blood samples. We correlated the percentage of MO-MDSC with PD at first imaging evaluation via Mann-Whitney test. We calculated the median value of MO-MDSC and compared the response rate (RR) as best response, progression-free survival (PFS) and overall survival (OS) between patients with MDSCs lower and higher median value, by means of chi-squared test for RR and Kaplan-Meier method for PFS and OS. A p-value <0.05 was considered statistically significant.

RESULTS

We included 22 patients who met eligibility criteria and signed informed consent. The median MO-MDSC value was higher in patients with PD (3,80 [95%CI:1,16-8,97] vs 1,45 [95%CI:0,79-1,91]; p=0,0242). Median value of MO-MDSC in overall population was 1,9. We observed tumor responses only in patients with higher MO-MDSC than the median (RR: 36% vs 0%; p=0,0316), longer PFS in the same group (median PFS: 7 months vs 1; HR=0,24; p=0,0136), but no significant differences in terms of OS (median OS: 15 vs 2; HR = 0,39; p = 0,0740).

CONCLUSIONS

In this analysis, high MO-MDSC percentage show a relevant role as predictive marker for primary resistance to ICIs, but these do not seem to be also prognostic. These findings suggest that the analysis of circulating MDSC could be used to select the NSCLC patients with a higher chance to benefit from an ICI. Moreover, new treatments targeting MDSCs could be developed for patients with higher levels of these cells in peripheral blood.

P041

IDENTIFICATION OF AN EPIGENOMICS SIGNATURE OF ENDOCRINE THERAPY-RESISTANT BREAST CANCER

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BACKGROUND-AIM

The Estrogen Receptor alpha (ERα), a key mediator of the estrogen signaling, is the primary oncogenic driver of 70% of breast cancer (BC) cases. Nearly one-third of the ERα-positive tumors develop endocrine therapy resistance (ET-R), manifesting disease relapse or progression. Besides genetic evolution, an altered epigenome might be responsible for the aberrant expression of genes playing a critical role in BC relapse. A well-characterized epigenetic regulator, the 5-methylcytosine mark (5mC), is currently guarded as a promising biomarker of ET-R, as the presence of methylated DNA fragments released by the tumor can be monitored in liquid biopsies from patient's blood and used to predict the clinical outcome.

METHODS

The methylation status of CpG sites (CpGs) across the genome has been investigated in BC with a Methylation Array approach, interrogating 850'000 CpGs. As in vitro models, 33 luminal-like, ERα-positive BCs cells, including Tamoxifen-resistant (TAM-R), Faslodex/ICI182,780-resistant (ICI-R) clones and parental sensitive cell derived from BT-474, MCF-7, T-47D and ZR-75.1 human BC subtypes were selected. RNA-seq profiling was used to correlate the methylation profile to the transcriptome of BC cells in the context of ET-R.

RESULTS

The 5mC landscape of BC cells has been investigated in depth, revealing the presence of hundreds of TAM-R and ICI-R-specific alterations in the DNA methylation pattern ($|Db| \geq 0.20$; $FDR \leq 0.1$) able to discriminate between resistant clones and parental cells. Differentially methylated CpGs are found in the promoter of well-characterized genes known to be related to ET-R, involved in key-signaling pathways, transcriptional regulation mechanisms, metabolic processes, membrane transports, invasiveness and epithelial–mesenchymal transition. A strong DNA-methylation signature has also been identified in the proximity of functional interactors and target genes of ERα required for BC cell proliferation.

CONCLUSIONS

For a selection of promising CpGs, data from TCGA BRCA patients show that the overall-survival (OS) probability of ERα-positive BC patients correlates with the expression of their putative regulated genes, a finding that was verified in our transcriptomic data, elucidating the presence of new potential determinants of resistance and putative biomarkers.

P042

PARP AND CHK1 INHIBITORS SYNERGIZE IN KILLING MYCN AMPLIFIED NEUROBLASTOMA VIA AN ATM-DEPENDENT PATHWAY

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BACKGROUND-AIM

Activation of oncogenes such as MYCN is associated with replication stress (RS) and DNA damage (DD), making MYCN-driven tumors addicted to the activity of proteins involved in RS and DD responses. Previously, we showed that PARP inhibitors (PARPi) enhanced MYCN-induced RS causing death via mitotic catastrophe, in MYCN amplified (MNA) and MYCN-overexpressing neuroblastoma (NB) cells. The simultaneous inhibition of the CHK1-dependent S-phase checkpoint exacerbated this phenotype. These data suggest that MYCN-induced RS could make MNA NB addicted to PARP and CHK1 activity, providing a rationale to test their combined inhibition for therapeutic purposes.

METHODS

MTS, trypan blue and comet assays were performed to measure cell growth, cell survival and DD accumulation in a panel of non-MNA and MNA cell lines treated with the PARPi olaparib and the CHK1 inhibitor (CHK1i) MK-8776. MNA cells were xenografted in nude mice, then treated with vehicle, olaparib (50 mg/kg), MK-8776 (25 mg/kg) or their combination. Tumor growth was measured by caliper or bio-luminescent imaging.

RESULTS

PARPi+CHK1i led to accumulation of DD and cell death in a synergistic and MYCN-dependent manner, in vitro. Consistently, PARPi+CHK1i significantly reduced the growth of MNA NB subcutaneous and orthotopic xenografts, and the growth of a Sonic Hedgehog-MYCN medulloblastoma model, with no toxicities.
IMR32, the most responsive MNA cell line, bears the putatively pathogenic p.Val2716Ala ATM mutation. Since PARPi are synthetic lethal with ATM loss in 11q-deleted NB, we asked whether ATM was involved in the responses to PARPi +CHK1i in MNA NB. CRISPR/Cas9-based genetic correction of mutant ATM showed that this mutation did not fully abolish ATM kinase function and was irrelevant for the biological responses to PARPi+CHK1i.
While ATM loss of function is expected to confer sensitivity to PARPi and CHK1i and indeed it sensitizes non-MNA NB cells to PARPi+CHK1i, ATM knock-out by CRISPR/Cas9 revealed that ATM is required for the proapoptotic effect of the combination, in MNA NB cells.

CONCLUSIONS

In conclusions, our data highlight a new potential chemo-free strategy to treat MYCN-driven tumors. Moreover, they shed light on the involvement of ATM kinase in shaping NB cell responses to PARPi+CHK1i depending on their genetic background.

P043

SOLUBLE ISOFORM OF ST2 IS A CARDIAC INDUCER OF COLLAGEN DEPOSITION IN AN EX VIVO MODEL OF OBESITY

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BACKGROUND-AIM

One of the main cardiac mechanosensor is the IL-33/ST2L complex composed by the allarmin IL-33 released into extracellular space by damaged cells during cardiac overload and its receptor, ST2L, which their binding promotes cell survival and anti-fibrotic signaling. After damage, to stop IL-33/ST2L signal, resident cardiac cells release sST2, the soluble isoform of transmembrane receptor of ST2, which sequesters IL-33, functioning as a decoy receptor and blocking its signal. In obesity, the sST2 become over express especially by hypertrophic fat cells. The aim of our study is to evaluate in an ex vivo model of obesity the role of sST2 in the promotion of collagen production by cardiac cells.

METHODS

Ten obese nondiabetic male Zucker rats (OB) (fa/fa-, 10 weeks of age) and 10 lean littermates (L) (Fa/+) at the age of 25 weeks were weighted and scarified according to Italian Ministry of Health Authorization (N°325/2015PR of 2015/04/05) and their heart and visceral adipose tissue (VAT) were collected for the analysis. VAT biopsies were cut in 30mg pieces and added into a co-culture system with H9C2 for 6-24 and 48 hours. At each time points, cells and culture medium were collected. In all samples were evaluated ST2 by ELISA and collagen were quantified by quantitative Sircol assay for measurement of both acid-soluble and pepsin-soluble collagens.

RESULTS

OB cardiac biopsies showed higher protein level of both ST2 and collagen than L rats ($p<0.02$ and $p<0.002$ respectively) and the rat weight positively correlates with ST2 cardiac protein level ($p<0.05$). Co-culture data demonstrated that OB VAT promotes ST2 releases in both cardiac and fat cells, resulting an incremental increase of sST2 release in cell culture medium than L VAT in time. sST2 quantification in correspondent H9C2 co-cultured lysate is more incremented in OB than L H9C2 after 48h.total collagen quantification in co-culture medium resulted higher in OB than L ($p<0.05$).

CONCLUSIONS

Our results demonstrated in a co-culture system that obesity promotes sST2 increase in the heart and this promotes collagen releases by cardiac cells, suggesting sST2 role in activation of pro-fibrotic signaling and detrimental heart remodeling.

P044

LIPOCALIN-2 MEDIATES THE SYNAPTOTOXIC EFFECT EXERTED BY OXYSTEROL-STIMULATED ASTROCYTES

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BACKGROUND-AIM

The presence of reactive astrocytes in Alzheimer's disease (AD) brain was demonstrated to correlate with neuronal loss and cognitive deficits. Evidence indeed supports the role of reactive astrocytes as mediators of changes in neurons, including synapses. Oxysterols (cholesterol oxidation products) are crucial for brain cholesterol homeostasis and we previously demonstrated that changes in the brain levels of various oxysterols correlate with AD progression.
In order to deepen the role of oxysterols in AD, we investigated whether they could contribute to astrocyte reactivity, and consequently impact on neuronal health.

METHODS

Two oxysterol mixtures were used, whose composition is based on our previous oxysterol quantification in mild (Early AD mix) and severe (Late AD mix) AD brain samples. Mouse primary astrocytes were treated with the oxysterol mixtures (10μM) at different time points. Mouse primary neurons were co-cultured with astrocytes previously treated with oxysterols. The transient knockdown of lipocalin-2 (Lcn2) gene was performed in order to investigate Lcn2 involvement in the synaptotoxic effect exerted by oxysterol-treated astrocytes.

RESULTS

Results showed that oxysterols present in the AD brain induce a clear morphological change in mouse primary astrocytes, accompanied by the upregulation of some reactive astrocyte markers, including Lcn2. Moreover, astrocyte conditioned media analysis revealed a significant increase in the release of Lcn2, cytokines, and chemokines in response to oxysterols. A significant reduction of postsynaptic density protein 95 (PSD95) and a concurrent increase in cleaved caspase-3 protein levels have been demonstrated in neurons co-cultured with oxysterol-treated astrocytes, pointing out that mediators released by astrocytes have an impact on neurons. Among these mediators, Lcn2 has been demonstrated to play a major role on synapses, affecting neurite morphology and decreasing dendritic spine density.

CONCLUSIONS

These data demonstrated that oxysterols present in the AD brain promote astrocyte reactivity, determining the release of several mediators that affect neuronal health and synapses. Lcn2 has been shown to exert a key role in mediating the synaptotoxic effect of oxysterol-treated astrocytes.

P045

INIBITORY EFFECT OF LDHA KNOCK-DOWN ON METABOLIC REPROGRAMMING AND C-MYC/H-RAS DRIVEN HEPATOCELLULAR CARCINOMA

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BACKGROUND-AIM

Hepatocellular carcinoma (HCC) is a multi-step process whereby abnormally proliferating cancer cells undergo a large metabolic reprogramming. Different metabolic alterations taking place in hepatocarcinogenesis are dependent on the activation of specific oncogenes, thus partially explaining the HCC heterogeneity. C-Myc oncogene overexpression frequently observed in human HCCs, leads to a metabolic rewiring toward a Warburg phenotype and production of lactate, thus resulting in the acidification of the extracellular space, which favors the emergence of an immune-permissive tumor microenvironment (TME). Here, we address the question as whether Lactate dehydrogenase alpha (Ldha) genetic ablation interferes with metabolic reprogramming and HCC development.

METHODS

We characterized by qRT-PCR, histochemistry and immunohistochemistry the metabolic reprogramming occurring in tumors induced in C57BL/6J male mice hydrodynamically co-transfected with c-Myc and h-Ras. Using the same experimental model, we also investigated the effect of Ldha inhibition – gained through the inducible and hepatocyte-specific Ldha knock-down - on cancer cell metabolic reprogramming, HCC number and size of HCCs, as well as TME alterations.

RESULTS

C-Myc/h-Ras driven tumors display a striking glycolytic metabolism suggestive of a switch to a Warburg phenotype. The tumors also exhibited enhanced activity of pentose phosphate pathway (PPP), the switch of glutamine to sustain glutathione synthesis instead of Tricarboxylic acid cycle (TCA) and the impairment of oxidative phosphorylation (OXPHOS). Ldha abrogation significantly hampered tumor number and size together with a clear inhibition of the Warburg-like metabolic feature. Loss of Ldha also caused a remarkable increase of CD4+ lymphocytes compared to Ldha wild type livers, thus preserving their anti-neoplastic effect.

CONCLUSIONS

These results demonstrate that Ldha deletion significantly impairs HCC development. and suggest the potential usefulness of targeting Ldha to enhance the efficacy of the current therapeutic options.

P046

THE ANDROGEN RECEPTOR/FILAMIN A COMPLEX AS A PLAYMAKER IN STROMAL AND EPITHELIAL PROSTATE CANCER CELLS.

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BACKGROUND-AIM

Prostate cancer (PC) still represents one of the most widespread malignancies in men. The androgen receptor (AR) plays a major role in its development and progression. It still represents one of the main targets in PC therapy. Most studies focus on the role of AR expressed in epithelial prostate cancer cells, while the functions of AR in CAFs are still neglected. In this scenario, the mechanism involving androgen/AR signaling in CAFs and its role in PC progression still remains elusive.

METHODS

Carcinoma's associated-fibroblasts (CAFs) have been prepared ex vivo from a cohort of patients affected by PC at different Gleason's score. The expression of AR and its interaction with signaling effectors involved in cell locomotion in stromal and epithelial PC cells has been investigated by biochemical and confocal microscopy approaches. Analysis of AR-mediated actions has been analyzed in 2D, co-culture, and 3D models.

RESULTS

In the extranuclear compartment of epithelial and stromal PC cells, AR interacts with Filamin A (FlnA) inducing cell invasion. In this context, androgen-stimulated CAFs move towards epithelial PC cells in 2D and 3D models. As a consequence, a significant increase in PC organoid's size is observed in 3D model. A stapled peptide, Rh-2025u, perturbs the androgen-induced AR/FlnA complex assembly, inhibits the invasion of CAFs, and strongly reduces the tumor size in androgen-treated 3D co-culture. Mechanistically, the androgen-induced AR/FlnA complex recruits β1-integrin and membrane type-matrix metalloproteinase 1, thus activating a protease cascade driving the extracellular matrix reshaping. The stapled peptide, Rh2025u, perturbs this mechanism.

CONCLUSIONS

Our findings propose new unexpected functions for AR expressed in CAFs and identify the Rh2025u peptide as a new drug to be used alone or in combinatorial therapies for a more tailored approach in PC treatment. Our research aims to discover targets, which could be "druggable" for inhibiting the invasiveness of PC cells. The stapled peptide, inhibiting the migratory phenotype of epithelial and stromal PC cells, stands as an excellent candidate in this field. With this approach, PC epithelial cells and their surrounding CAFs could be evaluated and targeted not as two distinguished compartments but as a single one.

P047

BIOLOGICAL CONDITIONS RELATED TO FRAILTY AND THEIR EFFECTS ON ADULT RENAL STEM CELLS CULTURED AS NEPHROSPHERES

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BACKGROUND-AIM

Frailty is a geriatric syndrome that can be associated to aging. It is defined as a state of increased vulnerability. The identification of frailty occurs through five criteria defined by Fried based on reduced physical and mechanical functions.

Frailty increases with aging and very often with progressive decline of renal function. There is a close association between frailty and CKD. In the scenario of kidney dysfunction few studies evaluate the relationship between frailty and renal function. As demonstrated for other tissues, even renal stem cell (RSC) exhaustion might play a role in renal aging The aim of our study is to evaluate the effects of biological conditions related to frailty on adult RSC and evaluate if these conditions are able to alter the RSC behavior.

METHODS

Whole blood from frail, non-frail and young subjects was collected and plasma was separated by centrifugation. Normal kidney tissues were obtained after nephrectomy in patients affected by renal tumors and nephrosphere (NS) cultures enriched in renal stem/progenitor cells were established and treated with 10% plasma of the enrolled subjects. Dissociated NS cells were stained with Annexin V/PI, Ki67 and γ-H2AX and analyzed by FACS. DNA damage was also investigated by immunocytochemistry.

RESULTS

We observed a statistically decrease of sphere forming efficiency (SFE), as evidence of self-renewal, in NS treated with plasma of frail subjects compared to the others. No differences were observed in cell proliferation and viability. By confocal microscopy analysis we observed both a statistically higher percentage of γ-H2AX positive cells and an increase in the number and fluorescence intensity of γ-H2AX foci per nucleus in NS cells treated with plasma of frail patients compared with others. We detected by FACS analysis the percentage of cells positive for γ-H2AX foci in dissociated NS cells and we confirmed a statistically higher percentage of cells with DNA damage in those treated with plasma of frail subjects.

CONCLUSIONS

Our findings show that plasma of frail patients have an effect on self-renewal and DNA damage of renal stem/progenitor cells. These results are not related to cell death or proliferation. Plasma of frail subjects may contain circulating factors able to induce alterations in renal stem/progenitor cell behavior.

P048

THERAPEUTIC EFFECT OF TG68, A NOVEL THYROID HORMONE RECEPTOR-β AGONIST, IN A MOUSE MODEL OF NAFLD

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BACKGROUND-AIM

Activation of hepatic thyroid hormone receptor ß (THRß) has shown encouraging beneficial effects on metabolic alterations, such as non-alcoholic fatty liver disease (NAFLD), obesity and diabetes. Here, we investigated the effect of TG68, a novel agonist of THRß, on fatty liver accumulation and liver injury in mice fed a high fat diet (HFD), and compared its effect with resmetirom (MGL-3196), a selective THRß agonist entering clinical phase III.

METHODS

C57BL/6 mice were fed HFD for 17 or 18 weeks, a time when all mice developed massive steatosis and steatohepatitis. Then, mice were given TG68 at a dose of 9.35 or 2.8 mg/kg for 2 or 3 weeks. As a reference compound, the same treatment was adopted using resmetirom at a dose of 10 or 3 mg/kg. Histological, histochemical and biochemical parameters were performed to investigate the effect of TG68. Gene expression was analyzed by qRT-PCR to assess the effect on THRß activation and lipid metabolism.

RESULTS

Treatment with TG68 did not modify body weight of the animals while it led to a significant reduction of liver weight, hepatic steatosis, serum transaminase and circulating triglycerides. qRT-PCR analyses showed activation of THRß as demonstrated by increased mRNA levels of Deiodinase I (Dio1) and Malic enzyme I (Me1), and change in lipid metabolism as revealed by increased expression of Acyl-CoA Oxidase I (Acox1) and Carnitine palmitoyltransferase I (Cpt1).

CONCLUSIONS

The results obtained with this novel THRß agonist unveiled a strong anti-steatotic effect coupled with amelioration of liver injury. The finding that TG68 exhibits the same beneficial effect of resmetirom provides an additional tool useful for the treatment of NAFLD.

P049

CELIAC DISEASE AND CANDIDA ALBICANS: AN INTRIGUING RELATIONSHIP

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BACKGROUND-AIM

Celiac disease (CD) is an autoimmune enteropathy resulting from an interaction between diet, genome, and immunity. Whilst many patients respond to a gluten free diet, in a substantive number of individuals the intestinal injury persists. Thus, other factors might amplify the ongoing mucosal inflammation in CD. Candida albicans is a commensal fungus that is well adapted to the intestinal life without inducing competitive interactions with other microbes or host immune system. However, specific conditions increase Candida pathogenicity. The hypothesis that Candida may be a trigger in CD has been proposed after the observation of similarity between a fungal wall component and two CD-related gliadin T-cell epitopes. However, despite being implicated in intestinal disorders, Candida may also protect against immune pathologies. The perturbation of bacterial community and chronic inflammation are favourable conditions to increased C. albicans pathogenicity contributing to CD pathology.

METHODS

To assess cytotoxic effects, we resorted to Caco-2 cells that were treated with digested gluten peptides, in presence or absence of Candida. For in vivo studies, we used a well-defined murine model of gluten sensitivity. Mice were inbred for 3 generations on a gluten-free diet and challenged with gliadin for 4 weeks. After the challenge, mice were infected intragastrically with C. albicans and evaluated for inflammatory parameters. In addition, we tested indole-3-carboxaldehyde (3-IAld), a microbial tryptophan catabolite known to restore gut homeostasis, in gluten-sensitized mice.

RESULTS

C. albicans potentiated the gliadin cytotoxic activity in Caco-2 cells by increasing the release of IL-15, an important marker of CD, and forming large cell-cell aggregates with more extensive cell damage activity. Similarly, Candida increased inflammatory immune response in gluten-sensitized mice. These effects involved mast cells (MCs) that define a pro-inflammatory microenvironment favourable to Candida virulence. Conversely, 3-IAld administration ameliorated intestinal injury via AhR/IL-22-dependent pathway activation and the modulation of MC function.

CONCLUSIONS

This study suggests that manipulating the inflammatory environment towards a tolerogenic state could improve the management of CD patients including C. albicans taming.

P050

ANDROGEN RECEPTOR: JOY AND PAIN OF PROSTATE CANCER.

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BACKGROUND-AIM

Prostate cancer (PC) is the second leading cause of deaths in men with cancer in Western countries. AR plays a pivotal role in tumour growth and therapies for PC usually reduce or inhibit the androgen production or displace the AR binding to their ligands. Most of the patients with advanced disease often exhibits drug-resistance and develop hormone-refractory PC (HRPC) for which there is no efficient therapy.
Despite the hormone-independence, HPRC expresses high level of AR, indicating that the receptor still exerts a role in tumour survival and progression, perhaps through its crosstalk with survival and metastatic programs. Many studies have supported the close relation between PC progression and epidermal growth factor receptor (EGFR).

METHODS

By combining different approaches, we have investigated the effect of androgens on the EGF-elicited responses in AR-positive HRPC cells.

RESULTS

Treatment of cells with 10nM of the synthetic androgen, R1881, does not affect proliferation of C4-2B cells. In contrast, they efficiently respond to EGF stimulation by showing an increase in both BrdU incorporation and invasiveness. 10nM R1881 drastically inhibits the EGF-mitogenic effect, leaving unaltered cell invasion. Androgen perturbation of the EGF-elicited mitogenesis is caused by a delay in EGFR degradation as well as a deregulation in the internalization of Ack1/clathrin complex. A persistence of the EGFR protein level upon combined treatment of cell with EGF and R1881 is recorded, simultaneously with a stronger and more persistent Erk activation. p27 is retained in nuclei and cell proliferation is halted. The AR antagonist, Casodex reverses these responses. In sum, androgens delay the EGF-induced down-regulation of EGFR, thus prolonging the EGFR-dependent signalling in HRPC cells.

CONCLUSIONS

Our findings highlight the opposite role of androgen/AR axis in PC. It fosters mitogenesis in hormone-sensitive cells, while is unable to drive this effect and even inhibits the EGF action in HRPC cells. These findings pave the way for the development of new therapies, which combine androgen-deprivation therapy (ADT) with the cyclical androgen supply to PC patients. This approach might significantly improve the efficacy, while reducing the patient's morbidity caused by the currently used ADT monotherapy.

P051

UNRAVELLING THE INHIBITORY ACTIVITY OF BOTULINUM NEUROTOXINS ON THE ENTERIC NERVOUS SYSTEM

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BACKGROUND-AIM

Botulism is a foodborne neuroparalytic syndrome caused by Botulinum Neurotoxin (BoNT). The syndrome is characterized by the inhibition of neurotransmitter release, causing flaccid paralysis and, in worst cases, death by respiratory failure. The effects BoNTs on somatic motor neurons has been largely characterized. On the other hand, little is known about the possible effects of BoNTs on the Enteric Nervous System (ENS), with the gut that is considered just a route of entry for the toxin. The ENS, also-called "second brain", has a central role in the control of enteric motility and secretions, but also in the protection against different threats thanks to its crosstalk with the enteric mucosal immune system and the gut microbiota. Therefore, we are investigating the possible effects of BoNTs intoxication on gut physiology from several points of view.

METHODS

All the experiments were made in vivo, with animals intoxicated with BoNTs type A or B. BoNTs proteolytical activity on ENS was assessed using immunofluorescence. The effects on peristalsis were evaluated using Charcoal Motility Test, while the systemic effects of the intoxication were evaluated with Compound Muscle Action Potential (CMAP) and Ventilation recordings. Fecal samples were collected for microbiome analysis. Oral intoxications with Salmonella typhimurium or Shigella flexneri were applied to evaluate Enteric Neuroimmune crosstalk efficiency.

RESULTS

We highlighted an exstensive proteolytic activity of both BoNTs serotypes on enteric cholinergic neurons. To understand the role of the small intestine in the disease onset, we treated animals with lowering doses of the two toxins, and we found a significant slowdown of the peristalsis even in absence of systemic symptoms. Given the impact of constipation on eubiosis, we are now investigating possible effects of the intoxication on gut microbiota homeostasis. Furthermore, we are approaching the evaluation of the effects on enteric neuroimmune crosstalk by the application of two models of oral infection.

CONCLUSIONS

In conclusion, the small intestine is not just a route to enter the body for BoNTs, but it represents their first important site of action. In fact, BoNTs already cause dysfunctions on gut phisiology at subclinical doses, with no evident symptoms of the disease.

P052

EPSTEIN-BARR VIRUS ENCODED EBNA2 DOWNREGULATES ICOSL THROUGH MIR-24-3P IN HUMAN LYMPHOMAS: IMPLICATIONS FOR RNA AIDED IMMUNOTHERAPY

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BACKGROUND-AIM

Immune checkpoints (IC) are a set of proteins which control T-cell responses so that our body is not harmed by the same cells which defend us. Cancer cells exploit these inherent IC controls by increasing PD-L1 or decreasing T-cell stimulatory proteins like ICOSL on their cell surface to compromise anti-tumor immunity. The discovery of ICs has revolutionized cancer immunotherapy. IC therapies use antibodies that inhibit or stimulate the ICs to enhance the body's immunological activity against tumors. The poor efficacy of immunotherapy using antibodies only calls for novel approaches. Given the altered expression of ICs and miRNAs in cancer, the latter could become fine candidates to increase the treatment efficiency by targeting ICs. Since EBV infected cancers have dysregulated expression of some ICs and miRNAs may be involved in regulating them, we have studied if and how EBV alters ICOSL and if miRNAs are involved in this process.

METHODS

Immunoblotting and flow cytometry were employed to test IC protein levels in recombinant EBV infected DLBCL and BL cells. Expression of predicted and validated ICOSL targeting miRNAs were tested by RT-qPCR in lymphoma cell lines. In vitro studies were performed on parental and EBNA2 transfected DLBCL, U2932.

RESULTS

We verified that EBNA2, a major transforming protein encoded by EBV, downregulated ICOSL and that miR-24-3p, an ICOSL targeting miRNA, is upregulated in EBNA2 transfected cells. In-silico analysis showed that miR-24-3p targeted ICOSL, therefore, we validated this by transducing B-lymphoma cells with ICOSL 3'UTR lentiviral construct and then transfecting the same cells with anti-miR-24-3p. By luciferase assay we confirmed that ICOSL is a direct target of miR-24-3p. Next, anti-miR-24-3p oligos were used to conclusively demonstrate that these anti-miR constructs suppressed miR-24-3p expression and consequently, ICOSL expression was restored.

CONCLUSIONS

We have previously shown that EBNA2 induces PD-L1 in B cell lymphoma. Here we find that the same viral protein downregulates ICOSL, usually important for T cell activation. Our findings provide new insights how miRNAs could be employed to regulate ICs and offer a novel miRNA/IC antibody combinatorial therapeutic approach for virus associated cancers.

P053

ABL2 IS INVOLVED IN TGFβ1-INDUCED INVASION AND INVADOPODIA MATURATION OF CLEAR CELL RENAL CELL CARCINOMA CELLS

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BACKGROUND-AIM

Renal cell carcinoma (RCC) accounts for about 30% of all adult malignancies with clear cell carcinoma (ccRCC) the most common variant. Up to 30% of newly diagnosed and 40% of previously treated ccRCC patients show metastatic disease. Tumor cells that disseminate and metastasize show F-actin rich cell protrusions with matrix-degradative activity known as invadopodia. Arg/Abl2 tyrosine kinase induces invadopodia maturation by phosphorylation of specific invadopodia components and, through cytoskeleton rearrangements, modulates invasion and metastasis of breast and prostate cancer. Invadopodia maturation is also induced by TGFβ signaling known to enhance RCC invasion. We have shown that Abl2 knockout increases TGFβ1 expression in fibroblasts. Here, we studied the role for Abl2 in the TGFβ-mediated ccRCC cell invasion and invadopodia maturation.

METHODS

Primary cell cultures, obtained from normal cortex and ccRCC tissue specimens, and 786-O ccRCC cell line have been used. We performed immunofluorescence analysis of invadopodia F-actin spots and matrix degradation, and 3D invasion assay in collagen I using Abl2 silenced 786-O cells treated or not with TGFβ1 and TGFβ-receptor inhibitor SB431542. 3D invasion assay was performed also with ccRCC primary cultures, treated or not with Abl2 inhibitor Imatinib. Western blot analysis on ccRCC primary cell cultures and cell line using anti-Abl2, anti-Phospho-Smad2/3, anti-Smad2/3 and anti-β actin has been performed.

RESULTS

In ccRCC primary cell cultures Abl2 protein expression was upregulated versus normal cortex and 3D invasion of ccRCC cultures was inhibited by Imatinib. Abl2 silencing in 786-O cells decreased 3D invasion, invadopodia matrix degradation activity and F-actin spots even after TGFβ1 treatment, suggesting a key role for Abl2 in both TGFβ-dependent and -independent ccRCC cell invasion and invadopodia maturation. Abl2 knockdown in 786-O cells did not affect Smad2/3 phosphorylation induced by TGFβ1 treatment, suggesting that Abl2 does not modulate TGFβ1/Smad pathway in TGFβ1-treated ccRCC cells.

CONCLUSIONS

Our studies show that Abl2 is necessary for TGFβ1-induced invadopodia maturation and for 3D invasion of ccRCC cells suggesting its putative role as therapeutic target in ccRCC metastatic disease.

P054

CNBP IS REQUIRED FOR LOCOMOTOR ACTIVITY IN DROSOPHILA MELANOGASTER THROUGH THE TRANSLATIONAL CONTROL OF ODC/POLYAMINE AXIS

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BACKGROUND-AIM

CNBP (ZNF9) is a conserved CCHC-type zinc finger RNA binding protein that regulates translation of various proteins, including that of ornithine decarboxylase (ODC), the rate limiting enzyme of polyamine metabolism. Microsatellite expansions of CCUG repeats in the first intron of the CNBP gene are associated to type 2 myotonic dystrophy (DM2). However, whether the clinical manifestations of DM2 are related to CNBP and polyamine metabolism is not known.

METHODS

We performed in vivo studies using several drivers to knock down or reconstitute dCNBP or dODC in different tissues and to address the specificity of the phenotype in muscles. We measured the climbing activity of flies and/or peristaltic waves of larvae. We analyzed the level of intracellular polyamines, in CNBP-deficient flies compared to the wt flies, and we performed biochemical studies in S2 cells or in an heterologous system (293t cells) to address the mechanisms by which polyamine synthesis is regulated by CNBP.

RESULTS

We show that depletion of dCNBP in fly muscles causes locomotor defects that are linked to an impaired polyamine metabolism. We demonstrate that, upon dCNBP depletion, the levels of ornithine decarboxylase (ODC) and polyamines are significantly reduced, and that ODC silencing phenocopies the dCNBP-dependent locomotor defects. We demonstrate that dCNBP controls polyamine metabolism by binding dODC mRNA and regulating its translation. Moreover, the dCNBP-dependent locomotor defects are rescued by either polyamine supplementation or dODC1 overexpression. Finally, we show a strong correlation between CNBP and polyamines levels in muscle cells from DM2 patients, which are both downregulated compared to heathy individuals.

CONCLUSIONS

These data illustrate a novel mechanism whereby dCNBP controls muscle function by regulating the ODC/polyamine axis. Together, our results provide the first evidence about dCNBP function in muscles diseases and its link to polyamine metabolism. These data may have relevant implications in CNBP-related pathophysiological conditions.

P055

THE NEUROPEPTIDE UROCORTIN 2 PROMOTES PERIPHERAL NERVE REGENERATION

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BACKGROUND-AIM

The neuromuscular junction (NMJ) consists of several non-myelinating perisynaptic SCs that physically cover the motor axon terminal (MAT) taking contact with the muscle fibre, to assure neurotransmission. Many pathological event may occur at this site leading to degeneration; however, the NMJ is characterized by a remarkable ability to recover from damage. In order to learn about the signallings that drive NMJ plasticity upon neurotransmission impairment and rescue within a short time window, our group has set-up a toxin-based model of reversible degeneration of the MAT. By this model based on the local injection of α-Latrotoxin (α-LTx), we profiled the transcriptomic changes occurring at the NMJ during MAT degeneration and the subsequent recovery.
Urocortin2 is a neuropeptide belonging to the corticotropin-releasing factor peptide family, involved in the physiological response to stress. It exerts its peripheral function a G protein coupled receptor, the Corticotropin Releasing Hormone Receptor 2 (CRHR2).

METHODS

The methods exploited are in vitro cultures, in vivo treatments, electrophysiological recording of evocked junction potential and Immunofluorescence

RESULTS

Among the differentially expressed transcripts, the messenger encoding for Urocortin2 (Ucn2) is strongly up-regulated soon after intoxication, when the pre-synaptic nerve terminal is undergoing degeneration, and then its levels decrease during the regenerative phase.
In vitro, CRHR2 is expressed both in SCs and on the axonal tip of cultured spinal cord motor neurons (MNs). Administration of exogenous Ucn2 promotes axon growth of primary MNs and, accordingly, co-incubation of Ucn2 and a selective receptor antagonist reverts this effect. In vivo, administration of the selective CRHR2 antagonist Astressin2B delays the functional and structural recovery of the NMJ upon α-LTx injection, while exogenous Ucn2 accelerates neurotransmission rescue of injured NMJs.

CONCLUSIONS

Overall, our findings speak in favour of a pro-regenerative action of Ucn2, and posit this molecule as a candidate pro-regenerative agent, to be tested in different models of peripheral nerve injuries.

P056

EIF5A HYPUSINATION PROMOTES COLORECTAL CANCER GROWTH THROUGH MYC TRANSLATIONAL ELONGATION

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BACKGROUND-AIM

Translation factor eIF5A undergoes a unique post-translational covalent modification called hypusination, which is catalyzed by two different enzymes: deoxyhypusine synthetase (DHPS) and deoxyhypusine hydroxylase (DOHH). Hypusinated eIF5A (Hyp-eIF5A) functions mainly by alleviating ribosome stalling in mRNA containing proline-rich motifs, facilitating their translational elongation. Recent studies have shown that eIF5A is overexpressed in different cancers, including CRC, where it correlates with poor prognosis. Previous reports documented the therapeutic efficacy achieved by using the specific DHPS inhibitor GC7, which impairs hypusination and limits the growth of several cancers. However, the mechanisms of action and the targets of Hyp-eIF5A are still largely unknown. In the present study we aim to show effects and mechanisms of the Hyp inhibition on CRC cell growth.

METHODS

To address the translational role of Hyp-eIF5A, we performed lentiviral or pharmacological inhibition of DHPS/eIF5A in different colon cancer cell lines, biochemical analysis of protein and mRNA, gene expression analysis, and polysome profiling.
In vivo studies were performed using the tumor prone APCMin/+ mice, a model of human familial adenomatous polyposis (FAP), and by performing xenograft studies in immunocompromised mice.

RESULTS

Inhibition of Hyp-eIF5A with GC7 or through knockdown of DHPS or eIF5A reduces cellular growth of different CRC cell lines. Gene expression analysis revealed that a relevant common regulator of the DHPS-modulated transcripts is the oncogene MYC, whose protein levels are downregulated in cells with impaired hypusination, while the mRNA and protein stability are not affected. We show that eIF5A regulates MYC translational elongation by alleviating ribosome stalling at five distinct pausing motifs in its coding sequence. Furthermore, we show that blockade of the hypusination axis elicits a remarkable growth inhibitory effect in preclinical models of CRC and significantly reduces the size of polyps in APCMin/+ mouse models.

CONCLUSIONS

Overall, we demonstrate that the tumor-promoting properties of Hyp-eIF5A are linked to its ability to regulate MYC elongation and we provide a rationale for the use of DHPS/eIF5A inhibitors in FAP or CRC therapy.

P057

POST-TRANSLATIONAL DOWN-REGULATION OF NRF2 AND YAP PROTEINS, BY TARGETING DEUBIQUITINASES, REDUCES GROWTH AND CHEMORESISTANCE IN PANCREATIC CANCER CELLS

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BACKGROUND-AIM

The intrinsic resistance of pancreatic ductal adenocarcinoma (PDAC) represents the main obstacle to treat this malignancy. In PDAC cells, the up-regulation of the antioxidant systems and the sustained cell proliferation are supported by the activation of Nrf2 and YAP proteins.

METHODS

We used three pancreatic cancer cell lines: PANC-1, MiaPaCa-2 and CFPAC-1. We evaluated their sensitivity to gemcitabine and CDDP through MTT test and measured the protein expression of Nrf2 and YAP and their target genes through western blot. We analyzed Nrf2 and YAP mRNA expression, through qRT-PCR, the oxidative stress with DCF-DA cytofluorimetric analysis and DUB-3 and OTUD-1 protein expression through western blot. In siRNA-silenced DUB-3 and OTUD-1 PANC-1 cells the expression of Nrf2 and YAP and the sensitivity to gemcitabine were analyzed.

RESULTS

PANC-1 cells were more resistant to gemcitabine and CDDP than MiaPaCa-2 and CFPAC-1 cells and had a higher protein expression of Nrf2 and YAP without a correlation with mRNA levels. According to a high Nrf2 level, oxidative stress was lower in PANC-1 cells. Total deubiquitinase activity was the same in the three cell lines, but protein expression of DUB-3 and OTUD-1 was higher in PANC-1 than in CFPAC-1 cells. DUB-3 and OTUD-1 silenced PANC-1 cells reduced cell proliferation and increased their sensitivity to gemcitabine.

CONCLUSIONS

Results demonstrated that Nrf2 and YAP deubiquitinases play a role in chemoresistance of pancreatic cancer and their silencing reduced cell growth of chemoresistant cells through the reduction of Nrf2 and YAP protein expression. These results suggest that DUB3 and OTUD1 can represent a suitable target to repress Nrf2 and YAP expression in PDAC cells.

P058

INTEGRATED ANALYSIS OF DNA METHYLATION AND CYTOKINES PRODUCTION IN MS PATIENTS

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BACKGROUND-AIM

Multiple sclerosis (MS) is a demyelinating disease with chronic inflammation, deregulation of cytokines gene expressions and resulting protein dysfunction. The epigenetic regulation of gene expression may play a pivotal role in MS pathogenesis. DNA methylation is an epigenetic alteration that efficiently controls gene expression and production via gene silencing and may significantly contribute to the MS development. Thus, we questioned whether DNA methylation of two key cytokines in the regulation of the inflammatory response, TNFα and IL-10, is modulated in MS patients, compared to healthy people, and if there is a direct association with their production

METHODS

This study was performed on 31 MS patients and 16 healthy subjects (HC). The blood samples were collected from all the subjects for DNA extraction, while serum was tested for cytokines levels. The methylation status of the TNFα and IL-10 gene promoter was confirmed using the pyrosequencing method and the average methylation index (MI) was calculated from the average percentage of methylation for all observed CpG sites. Cytokines levels in serum were evaluated using ELISA assay.

RESULTS

As we expected, the production level of TNFα increased significantly in the MS group compared to the HC and, in line with its anti-inflammatory role, the production of IL-10 is decrease in MS group in comparison with HC. MS patients showed a lower TNFα and IL-10 MI averages respect to HC. Our results suggest that methylation could be essential in the regulation of the TNFα production in MS patients and could participate in production of this cytokine, as seen in other autoimmune disease. While IL-10 gene methylation levels were not correlated with IL-10 observed production.

CONCLUSIONS

Epigenetic differences, such as lower level of TNFα and IL-10 methylation in MS patients compared to HC, can suggest their important role in MS pathogenesis. However, due to the complexity of TNF signaling and due to the mechanisms of IL-10 production and regulation by microRNA and circRNA, together with the epigenetic methylation, more research and discover are needed in the future.

P059

TARGETING MITOCHONDRIAL FISSION IS CYTOTOXIC AND EXERTS IMMUNE REGULATORY EFFECTS IN BORTEZOMIB-REFRACTORY MULTIPLE MYELOMA

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BACKGROUND-AIM

The strict dependence of multiple myeloma (MM) cells on the proteasome degradation system has provided the rationale for the development of proteasome inhibitors (PI) as anti-MM drugs. Unfortunately, the complex biology of MM cells inevitably triggers PI resistance, which has been linked to aberrant mitochondrial activity. Changes in mitochondrial dynamics are common in cancer and impact on various phenotypes, but their role in MM pathobiology has never been investigated.

METHODS

BZ resistant were generated by stepwise exposure of increasing BZ concentrations to parental cell lines. Mitochondrial structure was assessed by TEM. Cell viability was assessed by Cell Titer Glo or CCK8 assays, while apoptosis by Annexin V/7AAD staining. ROS were determined by H2DCFDA, while mitochondrial superoxide species by MITOSOX red staining. Daratumumab-mediated ADCC was assessed by FACS analysis of Far Red-labeled MM cells co-cultured with PBMCs.

RESULTS

A comparative TEM analysis between BZ sensitive and resistant isogenic cell lines highlighted rearrangements indicative of ongoing mitochondrial fission in the resistant counterpart. BZ resistant cells displayed upregulation of the main fission effectors-namely the GTPase Drp1, its active S616 phosphorylated form, the Drp1 receptor MFF-and of the inner mitochondrial membrane regulator OPA1, while down-regulation of fusion promoter MFN2. BZ treatment was instead accompanied by dose-dependent down-regulation of Drp1, MFF and OPA1 in PI-sensitive cells. Treatment with the mitochondrial division inhibitor mDIVI-1 reduced MM cell viability, even in co-culture with bone marrow stromal cells, increased ROS and mitochondrial superoxide species, and triggered apoptosis; conversely, mDIVI-1 was not cytotoxic to healthy PBMCs.

Finally, based on the emerging role of mitochondria in orchestrating immune responses, we investigated whether mitochondrial dynamics could impact on the antigenic profile of MM cells. Strikingly, BZ-resistant cells displayed lower CD38 surface expression than parental cells, and mDIVI-1 restored CD38 expression, increasing daratumumab-mediated ADCC.

CONCLUSIONS

These data shed light on novel targets for (immune)therapeutic interventions based on dysregulated mitochondrial dynamics in the MM PI-refractory setting.

P060

INDUCIBLE T-CELL COSTIMULATOR (ICOS) SIGNAL MEDIATES LYMPHOCYTE/MACROPHAGE INTERACTIONS DURING LIVER REPAIR

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BACKGROUND-AIM

Acute liver failure (ALF) resulting from viral infections, ischemia/reperfusion or drug toxicity has still high mortality in the absence of immediate intensive care and/or emergency liver transplantation. It is increasingly clear that inflammatory reactions are critical in the processes of parenchymal regeneration. Indeed, liver healing requires the switching of pro-inflammatory monocyte-derived macrophages (MoMFs) to a reparative phenotype. Nowadays, the mechanisms driving MoMFs phenotype switching are still poorly defined. In this study, we investigated the involvement of the inducible costimulatory molecule (ICOS) present on T-lymphocytes and of its ligand (ICOSL) expressed by macrophages in modulating liver repair.

METHODS

Hepatic injury was induced by carbon tetrachloride (CCl_4) poisoning in wild-type, ICOS (ICOS-/-) and ICOS (ICOSL-/-) knockout mice.

RESULTS

The role of the ICOS/ICOSL dyad was investigated during the recovery from acute liver damage induced by CCl_4. Flow cytometry of immune liver cells obtained from CCl_4-treated wild-type mice revealed that the recovery from acute liver injury associated with a specific up-regulation of ICOS in CD8+ T-lymphocytes and an increase in ICOSL expression involving CD11b high/F4-80+ hepatic MoMFs. Although ICOS deficiency did not influence the severity of liver damage and the evolution of inflammation, CCl_4-treated ICOS-/- mice showed delayed clearance of liver necrosis and increased mortality by ALF. These animals were also characterized by a significant reduction of hepatic reparative MoMFs due to an increased rate of cell apoptosis. An impaired liver healing and reparative MoMF loss was similarly evident in ICOSL-/- mice or following CD8+ T-cell ablation in wild-type mice. The loss of reparative MoMFs was prevented by supplementing CCl_4-treated ICOS-/- mice with recombinant ICOS (ICOS-Fc) which also stimulated full recovery from liver injury.

CONCLUSIONS

The data demonstrated that $CD8^+$ T-lymphocytes play a key role in supporting the survival of reparative MoMFs during liver healing through ICOS/ICOSL-mediated signaling. These observations open the possibility of targeting the ICOS/ICOSL dyad as a novel tool for promoting efficient healing following acute liver injury.

P061

THE ROLE OF NERVE GROWTH FACTOR IN GENDER-RELATED CANCERS.

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BACKGROUND-AIM

Prostate cancer (PC) and breast cancer (BC) are the most commonly diagnosed malignancies in men and women, respectively. Despite the significant efforts in early diagnosis and treatments, these cancers often progress acquiring drug-resistance. Although the role of steroid hormones and their cognate receptors in PC and BC progression is undeniable, other events are involved. Nerve growth factor (NGF) turned out to be a multifaceted molecule in quite divergent cells, including cancer cells. Many solid tumors exhibit derangements of NGF and its receptors, including the tropomyosin receptor kinase A (TrkA). This receptor is expressed in PC and BC, although its role in their pathogenesis and aggressiveness is still pending. We aim to identify the role of NGF and its dependent signaling in aggressiveness of PC- as well as triple negative BC (TNBC) - derived cells.

METHODS

Biochemical (Western blot and immunoprecipitation) and functional (proliferation, invasion and 3D culture) assays were used to link the activation status of NGF signaling with the aggressiveness of various PC and TNBC cell lines. Analysis of secretoma revealed an autocrine release of NGF and its forward loop in both cancer cell types. The effect of NGF/TrkA signaling impairment by neutralizing antibodies against NGF or TrkA inhibitors has been analyzed.

RESULTS

NGF robustly sustains the proliferation of these cells and stimulates their invasion. Such features, together with the release of the matrix metalloproteinase 9 (MMP-9), are responsible for the increase in the size of PC as well as TNBC-derived spheroids. Of note, PC and TNBC cells both release appreciable amounts of NGF. This finding, together with the observation that the cells express TrkA, indicates that an autocrine loop might sustain the proliferation and aggressiveness of these cancers. Neutralizing the NGF activity by a specific antibody shuts-down the biological activity of NGF. Inhibiting the activation of TrkA by the specific compound, GW441756, reverses the observed biological effects in both cancers.

CONCLUSIONS

Our results indicate the therapeutic potential of co-targeting NGF and TrkA in PC as well as TNBC. These findings might have an impact in gender-related cancers by providing new therapeutic opportunities in a specific and aggressive subset of PC or BC patients.

P062

TUMOR-TARGETING NIOSOMES FOR PHENFORMIN BRAIN DELIVERY IN MEDULLOBLASTOMA THERAPY

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BACKGROUND-AIM

Conventional cancer chemotherapy often fails in the clinical practice due to the development of resistance. In previous works, we have demonstrated that the antidiabetic phenformin plays an important role in the control of medulloblastoma proliferation, overcoming the pharmacological resistance to approved inhibitors. However, the clinical use of this biguanide is currently forbidden because of the risk of developing lactic acidosis, a dangerous condition due to the formation of lactate in the liver. Thus, one way to increase the therapeutic efficacy of phenformin with a reduction of its potential side effects would be its entrapment in tumor-targeting nanocarriers, with low affinity for hepatocytes. In this work, we have designed and prepared specific vesicular systems, called niosomes and characterized the effect of these phenformin-loaded nanovectors in medulloblastoma cells and in in vivo models. We have assessed their interaction with medulloblastoma tumor cells and their ability to maximize the pharmacological anti-cancer properties of the drug.

METHODS

In vitro cytotoxicity assay: Med1-MB cells were treated with niosomes at increasing concentrations and cell viability was estimated using Cell Titer Glo Luminescent cell viability assay (Promega). Fluorescent microscopy: For cellular uptake studies, cells were treated as described above, fixed and then observed under a fluorescence microscope. Western Blotting analysis: Cells were treated as described above. After harvesting, cells were lysed and the total protein was separated. Samples were visualized using enhanced chemiluminescence. 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 significantly enhances the concentration of the drug 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, while the liver concentrations were significantly lower.

CONCLUSIONS

Together, these data suggest that this novel niosome system could be an effective new treatment for brain cancer.

P063

THE MEK5/ERK5 PATHWAY IS A KEY MEDIATOR OF THE HEDGEHOG-GLI-DEPENDENT CELL PROLIFERATION IN MELANOMA CELLS

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BACKGROUND-AIM

Malignant melanoma is the deadliest skin cancer, with a poor prognosis in advanced stages. Available treatments improved the survival for this disease although they are not applicable to all patients and their long-term benefits are still unsatisfactory. We recently showed that the Mitogen-Activated Protein Kinase ERK5 plays a crucial role in melanoma, regulating cell functions critical for tumour development, such as proliferation. The Hedgehog-GLI signalling is a key regulator of tissue development, and its aberrant activation is involved in several types of cancer, including melanoma. It has been previously reported that Hedgehog-GLI pathway may support MAPK ERK1/2 activation, but the existence of a cross-talk with ERK5 has not been reported yet.

METHODS

Cell lines and patient-derived melanoma cells (wild type B-RAF: SSM2c; BRAFV600E: A375; NRASQ61R: MeWo) have been used. Activation of the Hedgehog-GLI pathway was obtained by silencing its negative regulator PATCH1 using lentiviral vectors expressing a specific shRNA (shPTCH1). ERK5 inhibition was achieved using ERK5 (JWG-071 and XMD8-92) or MEK5 (BIX02189) inhibitors or lentiviral vectors encoding shRNA specific for ERK5. Inhibition of Hedgehog-GLI pathway was achieved using GLI1/2 (GANT61) or SMO (MRT-92) inhibitors.

RESULTS

Activation of the Hedgehog-GLI pathway by genetic inhibition of PTCH1 increased the amount of ERK5 and MEK5 mRNA and protein. Chromatin immunoprecipitation showed that GLI1, the major downstream effector of Hedgehog-GLI signalling, binds to a functional non-canonical GLI consensus sequence at the MAPK7 promoter (the gene encoding for ERK5 protein). Interestingly, genetic inhibition of ERK5 reverted Hedgehog-GLI-sustained cell survival and proliferation in melanoma cells, as determined upon activation of the pathway by genetic inhibition of PTCH1. Finally, the combination of MEK5/ERK5 and Hedgehog-GLI pathway inhibitors determined synergistic effects in reducing colony formation with respect to single treatments.

CONCLUSIONS

ERK5 emerged as a key downstream effector of the Hedgehog-GLI signalling. Combined targeting of the ERK5/MEK5 and Hedgehog-GLI pathways may result in lower cytotoxicity and prevent resistance mechanisms frequently observed upon monotherapy in melanoma.

P064

VITAMIN D PREVENTS HIGH GLUCOSE-INDUCED LIPID DROPLETS ACCUMULATION IN ENDOTHELIAL CELLS BY DOWNREGULATING TXNIP

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BACKGROUND-AIM

Endothelial cells (ECs) represent a metabolic interface between blood and tissues and are pivotal to maintain vascular integrity. It is avowed that high glucose (HG) is detrimental for ECs, leading to redox imbalance partly because of the upregulation of TXNIP. Besides being involved in HG-induced ROS production, TXNIP is enrolled also in lipid metabolism. Little is known about fatty acid metabolism in EC. Since TXNIP was initially characterized as a target of Vitamin D (VitD) that exerts a protective effect in HUVEC through undisclosed mechanisms, the aim of this project was to study the effects of VitD in HUVEC exposed to HG, with a focus on lipid metabolism.

METHODS

HUVEC were cultured for 24h in medium containing physiological (5.5 mM) or high levels (11.1 and 30 mM) of D-glucose after TXNIP silencing with specific siRNA or in the presence of VitD (20 nM). N-Acetylcysteine (NAC, 5 mM) was used to prevent ROS production. ROS activity was measured using 2'-7'-dichlorofluorescein diacetate (DCFH) fluorescent probe. The amounts of some pro-/anti- oxidant proteins, among which TXNIP, and of proteins involved in lipid metabolism and transport (i.e. PPAR-γ, EDF-1 and CPT1A) was analysed by Western Blot. Triglycerides (TG) were quantified using TG assay and neutral lipids were visualized either with Bodipy 493/503 or with Oil Red O Staining. Fatty Acid Oxidation (FAO) was monitored by FAO assay in living cells.

RESULTS

HG in HUVEC is responsible for ROS increase through the upregulation of TXNIP. Moreover, HG-cultured cells upregulate PPARγ and EDF-1, recruited in lipogenesis, and downregulate CPT1A, leading to the impairment of lipid transport into the mitochondria. This is associated with a decrease of FAO. Furthermore, TXNIP silencing rebalanced the redox equilibrium and restored normal lipid content. It is noteworthy that the addition of VitD to HG-cultured cells prevented oxidative stress by downregulating TXNIP, therefore correcting lipid metabolism and storage.

CONCLUSIONS

In conclusion, VitD mimics the effects of silencing TXNIP, thus highlighting TXNIP as one of the targets of VitD in HG-cultured HUVEC.

P065

MAGNESIUM DEFICIENCY AND SKELETAL MUSCLE METABOLISM: A FOCUS ON LIPID HOMEOSTASIS

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BACKGROUND-AIM

Magnesium (Mg) deficiency is the most underestimated electrolyte imbalance in Western countries and is related to many dysfunctions. 25% of body Mg is located in skeletal muscle, where it is crucial for fibers relaxation. Hypomagnesemia is associated to muscle weakness and cramps and can contribute to oxidative stress, inflammation and age-related sarcopenia.
Our previous data in a murine model have shown that even a mild Mg deficiency is enough to induce a significant downregulation of genes involved in glucose and lipid metabolism, mitochondria dynamics and ATP production in skeletal muscle. We then investigated in vitro how Mg deficiency impacts on skeletal muscle lipid metabolism.

METHODS

We used C2C12 murine myoblasts that, under serum depletion, differentiate to multinucleated myotubes with contractile capacity. We exposed myotubes for 6 days to physiological (1 mM), mild low (0.5 mM) or severe low (0.1 mM) Mg concentrations. Protein expression was evaluated by western blot and immunofluorescence. Oxidative stress and metabolic parameters were studied with colorimetric and fluorescent assays.

RESULTS

Myotubes exposed to low Mg are characterized by a strong downregulation of the contractile protein myosin heavy chain (MHC) and by a reduced thickness in respect to control cells. Moreover, we observed a reduced deposition of lipid droplets (LDs) both in 0.1 mM and 0.5 mM Mg respect to 1 mM Mg. Coherently, we observed a reduced expression of Perilipin 2, involved in LDs formation.
Low Mg induces a stress response and, in particular, we observed nitric oxide (NO) overproduction in low Mg myotubes. The treatment with the NO synthases inhibitor L-NAME efficiently prevents NO overproduction. In the presence of L-NAME in low Mg cells, the LDs content returns comparable to the control condition.

CONCLUSIONS

Our 2D skeletal muscle model suggests a correlation between Mg deficiency-related stress response and metabolism. In particular, extracellular low Mg induces a significant nitric oxide-mediated stress response which influences lipid homeostasis.

P066

JAGGED1 RETROGRADE SIGNALLING SUSTAINED BY GAMMA-SECRETASE INHIBITORS CONFERS COLORECTAL CANCER RESISTANCE AGAINST CHEMOTHERAPY

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BACKGROUND-AIM

The members of Delta/Serrate/Lag-2 family are known proteins able to trigger the transactivation of Notch receptors, expressed on the signal-receiving cells. The dogma of the canonical Notch signal pathway is widely accepted, but it is not absolute. An emerging idea suggests that the nuclear-targeted intracellular domain of Jagged1 (Jag1-ICD) drives a "retrograde" signalling, in signal-sending cell.
Interestingly, in colorectal cancer (CRC), we have demonstrated that Jag1-ICD governs tumour progression and confers drug resistance.
Although surgical resection remains the gold standard of treatment for localized CRC, the availability of FOLFOX/ FOLFIRI regimens governs the current therapy for metastatic CRC (mCRC), but most of mCRC-bearing patients quickly develop drug resistance and recurrence.
Interestingly, γ-secretase inhibitors (GSIs) are used to inhibit Notch activation in several tumours, and have been progressively recognized as potential anticancer drugs, but their effects are quite controversial. We speculate that these GSIs-triggered conflicting effects, may be due to an intricate molecular scenario that exists inside the CRC cells, based on the expression of Jag1-ICD oncogene.

METHODS

Several human cell lines of CRC cancers were purchased from ATCC and cultured under standard conditions. Cells were treated with different GSIs and subjected to several in vitro assays, in order to evaluate the role of Jag1-ICD in proliferative, metastatic and chemoresistance events.

RESULTS

We demonstrate that the increased Jag1-ICD expression is strictly linked to mechanisms of multidrug resistance, leading to protection of CRC cells from apoptosis. Finally, we provide evidence about the synergistic effects induced by GSIs and oxaliplatin in both sustaining Jagged1 processing, via ERK, and inducing chemoresistance events in Kras-mut CRC cells.

CONCLUSIONS

Our data suggest that the current accepted therapies could play a controversial effect in CRC tumorigenesis, impairing the activation of Jagged1, which behaves like a novel oncogenic driver able to trigger an intrinsic reverse signalling with regulatory effects on tumour biology. Therefore, we indicate Jag1-ICD as a new molecular marker, useful to predict cancer progression, drug resistance and tumour relapse.

P067

“EPIGENETIC REPROGRAMMING OF CATB IN COLORECTAL CANCER: A VERSATILE PROGNOSTIC MARKER”

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BACKGROUND-AIM

Colorectal cancer (CRC) is the third most common tumor worldwide.Despite improvements in screening and patients' therapies,it can spread leading to metastatic disease.For CRC patients, surgery and chemotherapy have been considered the standard treatment, but those with metastatic lesions have poor prognosis and targeted therapy is new approach improving OS.In the last decades ECM has risen growing interest around its role in CRC tumorigenesis.The ECM has biochemical/mechanical properties, it is one of the proximal structures epithelial tumor cells destroy to favor migration. During CRC progression the ECM degradation is due to proteins like cistein- proteases, Cathepsins (CTSs).CTSs are intra/extracellular enzymes recently been identified as key elements in processes of tissue metastasis.It was investigated in CRC among CTSs, that CTSB enzyme is overexpressed and associated to the late stages with a short OS. Furthermore, its overexpression in/outside the tumoral cells offer the possibility of using its enzymatic activity as suitable tool in activating anticancer prodrugs in site- specific manner to avoid side effects.

METHODS

We designed and synthetized different prodrugs(doxorubicin based)targeted by CTSB and tested effects on cell vitality in combination with epimodulator (SAHA) in 2d and 3d CRC systems.

RESULTS

In this study there were analyzed CRC tissues, confirming CTSB hyperexpression in tumor in comparison to healthy counterpart, particularly in CRC right-side (with the worst prognosis) at late stages. It was demonstrated the epigenetic modulation of CTSB expression through use of some epi-modulators such as SAHA (HDACi) and JQ1 (BET inhibitor). The results revealed the potentiality to use epigenetic therapy in combination with prodrugs CTSB targeted in 2d and 3d CRC systems. In CRC cells and organoids, there was observed decrease in vitality suggesting that SAHA not only induces apoptosis but also increasing CTSB expression can promote a more efficient release of prodrug active part.

CONCLUSIONS

These data corroborate the idea to improve CRC therapy with the synergistic effect of epigenetic modulator and chemotherapeutic agent that can be addressed to the tumor cells avoiding side effects on healthy cells,thus improving CRC patients’ survival mostly in non-responder cases.

P068

LSD1/UTX INHIBITION PROMOTES METABOLIC REPROGRAMMING IN ANDROGEN-DEPENDENT AND INDEPENDENT PROSTATE CANCER

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BACKGROUND-AIM

Aberrant androgen receptor (AR) activities is the primary cause of the development and progression of prostate cancer (PCa) and castration-resistant PCa (CRPC). Androgen signaling regulates the main cellular processes such as metabolism supporting tumor growth through its transcriptional activity. AR expression and activity can be epigenetically regulated by lysine demethylases (KDMs) such as lysine-specific demethylase-1 (LSD1) and lysine-specific demethylase 6A (UTX), both overexpressed in PCa and CRPC.

METHODS

Here, we investigated the association between metabolism, epigenetic activity, and hormone signaling in different forms of PCa. Evaluating proteins modulation with proteomic assay and lipid content with lipidomic analysis, secondary to LSD1/UTX inhibition.

RESULTS

We showed that epigenetically LSD1/UTX inhibition, with the dual-KDM inhibitor MC3324, induces significant antiproliferation and apoptosis in androgen-responsive and -unresponsive PCa systems due to an increase in H3K4me2 and H3K27me3. Epigenetic LSD1/UTX inhibition downregulates AR at both transcriptional and non-transcriptional level, potentially avoiding the inefficacy of androgen deprivation therapy caused by androgen insensitivity of AR. Interestingly, LSD1/UTX inhibition disrupted mitochondrial ATP production and mediated a lipid plasticity which affects the phosphocholine class, an important structural element for the cell membrane in PCa and CRPC.

CONCLUSIONS

Disrupting bioenergy production and reprogramming the lipid component using this multi-target epigenetic approach may overcome mechanisms of therapeutic resistance in PCa.

P069

IMMUNOSENESCENCE AND INFLAMMAGING IN MULTIPLE SCLEROSIS: MULTIPARAMETER PHENOTYPING OF PERIPHERAL BLOOD

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BACKGROUND-AIM

Inflamm-aging represents a chronic low-grade inflammation that is particularly described with advanced age. It is also now considered one of the risk factors predisposing to the development of neurodegenerative diseases, including multiple sclerosis. As recently described, in multiple sclerosis, accelerated cellular senescence can drive the progression of the disease by promoting chronic inflammation, loss or alteration of immune, glial and neuronal functions, failure of remyelination, weakened blood-brain barrier integrity and neurodegeneration.

METHODS

In collaboration with the Institute of hematology and blood transfusion, Prague, and the Section of Neurology, Department of Medicine and Surgery, University of Perugia, we have isolated mononuclear cells from peripheral blood of multiple sclerosis patients early after the diagnosis of the disease and before medical treatment. The aim was the use of the 'Cytek Aurora' full spectrum technology to build a multiparameter 'Inflamm-aging panel' through a wide array of new fluorochrome combinations in order to deliver high-resolution data.

RESULTS

Although preliminar, the Cytek technology helped us to confirm the immune 'senescence' of immune peripheral cells in young patients. The immune panel confirmed the presence of activated monocytes, the aged phenotype of NK cells, the increased presence of CD8+ memory cells, and the increased percentage of CD8+TEMRA cells.

CONCLUSIONS

Since we have shown previously that in multiple sclerosis, low metabolic activity of enzymes as IDO may lead to low-grade inflammation, the characterization of the 'aged' immune cells in peripheral blood may represent a promising new technology to investigate for early markers of diagnosis.
In addition, it may open the way to mechanistic studies to understand the pathophysiology of the disease, or the mechanism of action for future therapeutic interventions.

P070

RIPK1-TARGETING IN CANCER: MODULATION OF PROGRAMMED CELL DEATH PROCESS FOR CANCER TREATMENT

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BACKGROUND-AIM

Proliferation, differentiation, and cell death are physiological events responsible for the maintenance of cellular homeostasis. Principal characteristic of tumor cell is to escape programmed cell death (PCDs) processes, going to a consequent malignant transformation. The recent discovery of a new form of programmed necrosis called necroptosis has opened the way to develop pro-necroptotic drugs for anti-cancer therapy. The process can be triggered by a variety of stimuli and requires the activation of receptor-interacting protein (RIP) kinases RIPK1 and RIPK3, as well as mixed lineage kinase domain-like protein (MLKL). Until now, little is known about the biological relevance of RIPK1 in cancer. A major goal is to understand "how" RIPK1 triggers different cellular pathways leading the cells to make a critical decision either to survive or die.

METHODS

With a novel multi-acting pan-SirT inhibitor, a time-course experiment was performed in a monocytic leukaemia cell line and the percentage of cells positive to PI was measured. To gain further mechanistic insights, RIPK1 expression was evaluated by Western blot analysis. The expression of necrosome formation was investigated by studying RIPK3 and MLKL protein expression. To close the gap between cellular function and transcriptomics, RNA-Seq analysis was performed.

RESULTS

The molecular mechanism of the compound highlights its ability to directly target RIPK1 within the molecular complex activated determining a decrease in RIPK1 protein expression while no substantial modulation was observed for RIPK3 and MLKL. To generally describe the pathways of genes obtained from RNA-Seq, the TOPPGENE database contains abundant information that can help understand the biological functions.

CONCLUSIONS

The reduced expression of RIPK1 protein assumes the existence of an alternative necroptotic cell death pathway with consequent reduction of the inflammatory response. This phenomenon, with less adverse effects than necroptosis, needs to be more investigated by making the impaired cell death process a pharmacologically druggable event. However, our findings support the key role of the SirT inhibitor as an epi-drug fully in line with the concept of epigenetic therapy, which aims to restore the correct execution of altered and silenced cellular pathways.

P071

POTENTIAL MECHANISMS OF ENDOCRINE DISRUPTOR BISPHENOL A (BPA) ON HUMAN COLON CANCER CELLS

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BACKGROUND-AIM

Bisphenol A (BPA) belongs to organic synthetic compounds widely used in the packaging for drinks and food as the manufacture of epoxy resins and polycarbonate plastic. BPA is considered an environmental pollutant that can leach into food and water, then it can be detected in human fluids. There is growing evidence that BPA may have a harmful effect on human health acting as an endocrine disruptor. However, although the gastrointestinal tract is directly involved with the oral ingestion of BPA, its influence on colorectal cancer carcinogenesis remains unexplored. Herein, we investigate the effects elicited by BPA in different colon cancer cell lines.

METHODS

By combining different proliferation assays (WST-1 analysis, BrdU incorporation) we analyzed the effect of BPA on cell proliferation of two colon cancer cell lines at a different stage of aggressiveness (Caco-2 and HCT-116). Additionally, by western blot analysis, we have investigated some of the key signaling effectors involved in this process. By using specific inhibitors of the signaling transduction, we suggest an interesting new putative role for the Epidermal growth factor and Nerve growth factor receptors (EGFR and Trk A, respectively) in the BPA mechanism of action.

RESULTS

BPA triggers cell proliferation in both cell lines at nanomolar concentrations through the down-regulation of p27 expression and upregulation of cyclin A expression. Moreover, BPA induces rapid activation of signaling effectors involved in the proliferation. Selective TrkA inhibitor, GW-441756, and EGFR-selective inhibitor, ZD 1839 (Iressa), completely abolishes BPA-induced TrkA and EGFR phosphorylation. Both the inhibitors significantly revert BPA-triggered activation, inhibiting ERK, Akt, P90 RSK, and GSK-3 α/β phosphorylation.

CONCLUSIONS

Altogether, these data suggest that environmental concentration of BPA exposure promotes cell proliferation of both colon cancer cell lines. In addition, TrkA and EGFR may play a key role in BPA-induced proliferation. These studies require further insight into the molecular mechanism to understand how BPA induces these effects. Furthermore, 3D models using colon no cancer cells and colon cancer cells by human biopsies will offer further insights in understanding the molecular mechanism of BPA.

P073

EXPLORING NEW FUNCTIONS OF THE NIJMEGEN BREAKAGE SYNDROME GENE IN CEREBELLAR DEVELOPMENT

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BACKGROUND-AIM

The DDR-defective Nijmegen Breakage Syndrome (NBS) is due to hypomorphic mutations of the NBS1 gene, whose protein orchestrates complex cellular responses to DNA damage and replication stress. Albeit the clinical phenotype of NBS has been mostly ascribed to DDR impairment, the aetiology of the neurological features, such as microcephaly, are still poorly understood.

The neurological features of NBS patients have been modelled through central nervous system (CNS)-restricted NBS1-KO in mice, which manifests phenotypes such as microcephaly, ataxia and cerebellar hypoplasia. Of note, mice with CNS-conditional KO of the SHH pathway, the main driver of granule cell progenitor (GCP) expansion during cerebellar histogenesis, show similar phenotypes, suggesting a possible overlap between NBS1 and the SHH pathway. This is partially supported by our recent studies, which indicated an epistatic function of NBS1-KO on the SHH pathway during cerebellar development and tumorigenesis. This prompted us to generate the hypothesis that NBS1 has a new direct role on SHH pathway regulation and consequently on cerebellar development.

METHODS

To address this hypothesis, we generated and analyzed a mouse model with a GCP-restricted NBS1 inactivation (NBS1GCP-Δ). GCPs were characterized by gene expression analysis by Microfluidic CARD, protein expression by Western Blot analysis and Immunofluorescence assays.

RESULTS

Our in-depth characterization of the new animal model highlights how NBS1 depletion in GCPs is sufficient to induce an aberrant formation of the cerebellar cortical architecture; this is reflected in a reduced cerebellar size with impaired proliferation and differentiation of GCPs, coupled with a severe alteration in the activity of the SHH pathway in an in vivo, ex vivo and in vitro context.

CONCLUSIONS

Our results support the hypothesis that NBS1-KO downregulates the SHH pathway in developing GCPs, thus providing new insights into the consequences triggered by NBS1 deletion on cerebellar development. More in general, our results offer a novel interpretative frame for the extreme vulnerability of the nervous system to genetic defects in DDR proteins, as revealed in DDR-defective syndromes.

P074

INACTIVATION OF THE NIJMEGEN BREAKAGE SYNDROME 1 (NBS1) PROTEIN ABROGATES SHH-DEPENDENT MEDULLOBLASTOMA BY ALTERING PRIMARY CILIUM DYNAMICS

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BACKGROUND-AIM

Medulloblastoma (MB) is a pediatric tumor that stems from the transformation of granule cell progenitors (GCPs) in the developing cerebellum. Mutations in DNA Damage Response (DDR) genes have been associated with MB, indicating a role for DDR proteins in its insurgence. NBS1 is a member of the MRN complex, essential in the maintenance of genome integrity and in the DDR. We recently demonstrated a dual function of NBS1 in MB genesis, which is contingent to its genetic dosage. In particular, neuronal-specific NBS1-KO completely abrogates SHH-driven tumorigenesis. However, it also severely impacts on cerebellar development blunting the possibility of further investigations. Of note, derangement of the cerebellar architecture is associated with a strong impairment of the SHH pathway, suggesting that NBS1-KO is epistatic to this pathway. This prompted us to more specifically address the relationship between NBS1 and the SHH pathway in GCPs, the cells responsible for SHH-dependent tumorigenesis.

METHODS

We generated a new model with GCP-restricted inactivation of both NBS1 and Ptch1, the SHH inhibitory receptor. We monitored the survival of the animals and performed histomorphometric analyses on cerebella. We used confocal microscopy to analyze the morphology/dynamics of the primary cilium (PC) on cerebellar tissue sections, and NBS1-KO mouse and human cell lines. Moreover, we analyzed PC structure/functionality in GCPs in vitro and in vivo, exploiting mouse models with NBS1 conditional alleles and cell-specific CRE drivers.

RESULTS

We demonstrated that NBS1 loss in GCPs abrogates Ptch1-dependent MB formation and impairs SHH pathway in a cell autonomous manner, confirming that NBS1-KO is epistatic to this pathway. Mechanistically, NBS1-KO leads to severe alterations of the PC, a pivotal organelle for SHH signaling, mostly affecting its length and shape. Moreover, NBS1 ablation causes deferred cilium disassembly and delayed cell cycle progression. Newsworthily, NBS1 localizes not only at the centrosome, but also at the base of the PC (basal body), further strengthening the hypothesis of its new function on PC regulation.

CONCLUSIONS

Our data reveals a novel function for NBS1 in regulating PC morphology, dynamics and function through which it could control SHH-dependent cerebellar development and tumorigenesis.

P075

MIR-181A-5P, MIR-324-5P, MIR-451A, SMN2 FULL-LENGTH LEVELS AND SMN2 COPY NUMBER: A MOLECULAR SIGNATURE FOR SPINAL MUSCULAR ATROPHY PATIENTS

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BACKGROUND-AIM

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by second motor-neuron degeneration, ranging from severe to mild forms. Experimental evidence support the pathogenic role of skeletal muscle in SMA. Irrespective of the severity, patients have the homozygous loss of the SMN1 gene. SMN2 is a hypomorphic allele, producing insufficient protein levels, and having variable copy number in patients (two-to-four). It is the only consistent phenotypic modifier, grossly related with disease severity. The amazing results of the available treatments, even more striking in presymptomatic patients, coupled with newborn screening programs ongoing, have made compelling the need of prognostic biomarkers to predict the progression trajectories of patients identified at birth. Beside the SMN2 products, few SMN-independent biomarkers have been evaluated so far, including some miRs.

METHODS

We performed whole miRNome analysis of muscle samples of patients and controls (biopsies and cultures) to identify deregulated miRs. The levels of muscle differentially expressed miRs were evaluated in serum samples (51 patients and 37 controls), alongside with SMN2 copy number, SMN2 full-length transcript levels in blood and age of patients. These parameters were integrated in a composite severity score (SMA-score).

RESULTS

Over 100 miRs were differentially expressed in SMA muscle; 3 of them (hsa-miR-181a-5p, -324-5p, -451a; SMA-miRs) were significantly upregulated in the serum of patients. The integration of SMA-miRs in the SMA-score increased the prediction of the severity up to a correlation coefficient of 0.87 ($p<^{10-5}$).

CONCLUSIONS

Our data suggest the primary involvement of skeletal muscle in SMA pathogenesis; the SMA-miRs are likely actively released in the blood flow even if their function and target cells require to be elucidated. If the accuracy of the SMA-score will be proven in replicative studies, it might be routinely evaluated to define the prognosis of patients identified presymptomatically.

P076

QUERCETIN PRE-TREATMENT ON DOXORUBICIN-INDUCED FIBROBLAST SENESCENCE REDUCE OSTEOSARCOMA CELLS GROWTH AND MIGRATION.

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BACKGROUND-AIM

Cellular senescence (CS) is commonly a tumour-suppressive mechanism, but it can also promote cancer progression, by secreting senescence-associated secretory phenotype (SASP) factors. Potential strategies for mitigating the damaging effects of CS include reducing the number of senescent cells or interfering with the SASP. In this study, we have first analysed the ability of Quercetin to reduce the induction of CS in Wi-38 fibroblasts by Doxorubicin, a chemotherapy used in the treatment of Osteosarcoma (OS), a malignant bone tumour with a high mortality rate, due to metastatic spread and therapy toxicity. Moreover, we have also studied the effect of conditioned media (CM) from Doxo-induced senescent fibroblasts (DSF) and Quercetin-pretreated DSF (QDSF) to OS cells growth and migration.

METHODS

WI-38 cells were incubated with/without Quercetin 40 μM for 24h and then treated with Doxo 50nM for 48h. 72h after Doxo-treatment, the senescent phenotype was evaluated by analyzing the expression of senescence-associated β-galactosidase activity (SA-β-gal), cell cycle arrest markers, and senescence-associated heterochromatin foci (SAHF). Moreover, CM from DSF (DSF-CM) and QDSF (QDSF-CM) were used to treat U2OS cells (OS). CM coming from no treated fibroblasts was used as control.

RESULTS

QDSF shows a significant reduction of SA-β-gal activity, cell cycle arrest markers, and SAHF presence compared to DSF. Quercetin increases the cells' antioxidant capacity and reduces ROS production, partially protecting the cells from Doxo-induced damage and senescence. Further, we have observed that U2OS cells treated with DSF-CM significantly increase their growth and migration, while those treated with QDSF-CM behaved like the control cells.

CONCLUSIONS

The effects of SASP from therapy-induced senescent (TIS) fibroblasts on tumor microenvironment remains yet to be clarified. However, our study shows that DSF-CM significantly increased U2OS proliferation and migration, promoting aggressive behaviour, while QDSF-CM does not induce the same aggressiveness. Our results might pave the way for the use of Quercetin to contrast the adverse effects of TIS on OS microenvironment and might be an excellent strategy to reduce therapy toxicity and metastatic spread.

P077

DEPRESSION AND CARDIOVASCULAR RISK IN OBESITY: IMPACT OF PCSK9 AND BDNF ON ADIPOCYTES.

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BACKGROUND-AIM

Obesity, raising Worldwide, increases the susceptibility to cardiovascular diseases (CVDs) and mood disorders. Among these, depression (affecting ~10% of the population) enhances the CVD risk and it is approximately twice as prevalent in women. Obesity, depression and CVDs often come hand in hand, although a mechanistic link among these three conditions remains not well defined. This uncertainty makes imperative to unravel molecular pathways beneath these liaisons.

METHODS

In 743 obese individuals, participating in the cross-sectional SPHERE (Susceptibility to Particle Health Effects, miRNAs and Exosomes) study, we evaluated possible mediators of the link between depression and obesity (proprotein convertase subtilisin/kexin type 9 (PCSK9) and Brain-Derived Neurotrophic Factor (BDNF)). We have deepened the molecular mechanisms contributing to this association by taking advantage of an in vitro model of human adipocytes (SW872 cells).

RESULTS

In the SPHERE cohort, PCSK9, a key-regulator of cholesterol, mediated 11% of the relationship between depression and insulin resistance, a CVD risk factor. This association was lost in carriers of the loss-of-function PCSK9 R46L variant, confirming a possible causal role for PCSK9 in the link between depression and insulin resistance. Since SPHERE cohort comprises obese individuals, the effects of PCSK9 on SW872 cells were investigated. The silencing of PCSK9 led to a phenotype toward a raised adipocyte differentiation process. Since BDNF Val66Met human polymorphism is involved in the onset of depression and CVD risk, as well as in the adipose tissue pathophysiology, we measured circulating BDNF levels in the SPHERE cohort. Circulating BDNF was negatively associated with depression (BDI-II score), while positively associated with insulin and HOMA-IR, an index of insulin resistance, in females. Treatment of SW872 cells with ProBDNFMet synthetic peptide impaired adipogenesis and the insulin signaling pathway.

CONCLUSIONS

PCSK9 and BDNF may share biological mechanisms underlying the association between depression and insulin resistance. This suggests how they may be intertwined in modulating CV risk factors in the presence of an obesity-driven depressive-like phenotype.

P078

AN INTEGRATIVE IN SILICO DATA ANALYSIS DISCLOSES A PREVIOUSLY UNIDENTIFIED MOLECULAR SUBTYPE OF COLORECTAL CANCER POTENTIALLY ELIGIBLE TO IMMUNE CHECKPOINT IMMUNOTHERAPY

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BACKGROUND-AIM

Historically, the molecular classification of colorectal cancer (CRC) was based on the global genomic status, which identified microsatellite instability (MSI-H or MIN) in mismatch repair deficient (dMMR-MSI-H) CRC, and chromosomal instability (CIN) in MMR proficient (pMMR-MSS/MSI-L) CRC. With the introduction of immune checkpoint inhibitors (ICIs), MIN/CIN classification regained momentum as the dMMR-MSI-H condition predicted sensitivity to ICIs in clinical trials, possibly due to both high tumor mutation burden (TMB) and high levels of infiltrating lymphocytes. Conversely, pMMR-MSS/MSI-L CRC are mostly resistant to ICIs.
To better understand the relationship between MIN/CIN, and eventually discover additional CRC subgroups relevant for therapeutic decisions, we performed an integrative in silico dataset analysis.

METHODS

Copy number variation (CNV), mutational, transcriptomic, weighted gene co-expression network analysis (WGCNA), and base substitution fingerprints analysis were performed using R programming language, on 520 CRC samples' data downloaded from the TCGA database.

RESULTS

By performing unsupervised hierarchical clustering analysis of CNVs versus hypermutation status (HM), we identified two main tumor clusters: (A) CRC with few CNVs enriched in HM samples and (B) CRC with a high number of CNVs mostly including non-HM samples. Within (A), we noted a group of samples (7.8% of the entire dataset) with low CNVs and low TMB, which shared clinical-pathological features with HM CRC (HM-like CRC). Analyses of the mutational profiles and base substitution fingerprints indicated that HM-like tumors are distinct from both HM and non-HM CRC and are likely to develop and progress through different genetic events. WCGNA further highlighted differences amongst the groups at the gene expression level and revealed HM-like tumors bear high expression of immune response genes. Analysis of immune/inflammatory infiltration signatures confirmed that HM-like tumors are characterized by T and NK cells signatures similar to HM samples.

CONCLUSIONS

Our work highlights HM-like tumors as a previously unidentified CRC subgroup potentially responsive to ICIs. If validated with additional in silico and experimental work, these finding can lead to expanding the fraction of patients eligible to ICI treatment.

P079

THE MULTIFACED ROLE OF MAML1: NOT ONLY A TRANSCRIPTIONAL COFACTOR. NEW INSIGHT IN CANCER BIOLOGY

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BACKGROUND-AIM

In mammals, Maml1 belongs to a family of proteins, which act as transcriptional coactivator for Notch signalling, an evolutionarily conserved pathway. Maml1 has been recently shown to act as a coactivator in other cell signalling pathways, such as p53, Wnt, Hedgehog and Hippo in a Notch-independent manner. The E3 protein ubiquitin ligase (E3) Itch, binds different proteins diverting most of them to a proteasome/lysosome-dependent degradative pathway. In Hedgehog context, Itch activity is enhanced by the protein Numb to induce Gli1 ubiquitination and proteasome degradation. Moreover, Numb interacts with Itch to promote degradation of Notch intracellular domain.

METHODS

We performed immunoprecipitation and ubiquitination assays both in vitro and ex vivo to assess the post-translational role of Maml1 and to identify Itch post-translational modification as a result of Maml1 interaction.

RESULTS

Our data suggest a potential role of Maml1 in the post-translational regulation of Gli1 and Notch1, preventing their degradation mediated by Itch. Besides a direct role as transcriptional coactivator, we demonstrate that Maml1 is able to control the expression of Itch-target proteins at post-translational level, by inhibiting directly Itch activity, through its C-terminal domain, still poorly characterized. So far, scientific research on Maml1 has been generally focused on its activity as a transcriptional coactivator, while overlooking its role in the post-translational regulation. We have identified the molecular mechanism through which Maml1 is able to modulate Itch functional activity on target proteins, by regulating its ubiquitination status.

CONCLUSIONS

A thorough understanding of the molecular mechanism mediated by Maml1 to regulate Itch activity through Maml1 C-terminal domain, might lead to novel future therapeutic approaches directed against cancer. Maml1 and Itch are both key molecules that connect different signalling pathways. Therefore, the ability of Maml1 to regulate Itch activity could have an impact in controlling the force of several pathways, such as Hedgehog and Notch, in deregulated pathological contexts. This could help to set out new therapeutic approaches based on the dual role of Maml1 and adding a piece in the understanding of tumour biology.

P080

A NOVEL SELECTIVE AND HIGH-AFFINITY CB2 AGONIST SHOWS IN VITRO ANTI-INFLAMMATORY PROPERTIES

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BACKGROUND-AIM

The endocannabinoid system (ECS) plays a role in immune modulation. The immunosuppressive effects exerted by cannabinoids are mostly mediated by cannabinoid receptor 2 (CB2), whose expression on leukocytes is higher than CB1, which is mainly localized in the brain. In fact, targeted CB2 activation could mitigate excessive immune responses, avoiding CB1-related psychoactive effects. Herein, we evaluated in vitro the biological activity of a novel, selective and high-affinity CB2 agonist, called JT11, studying its potential CB2-mediated anti-inflammatory role.

METHODS

Trypan Blue and MTT assays were used to test the cytotoxic and anti-proliferative effect of JT11 on Jurkat cells. Propidium iodide staining and western blot analysis of PARP-1 cleavage were run to assess its pro-apoptotic activity. Analysis of ERK1/2 levels after CB2 knockdown by siRNA was used to further verify the selectivity of JT11. At last, we observed its impact on LPS-induced ERK1/2 and p65-NF-κB activation and TNF-α, IL-1β, IL-6 and IL-8 release in peripheral blood mononuclear cells (PBMCs) from healthy donors, by western blot and Luminex technology, respectively.

RESULTS

JT11 regulated cell viability and proliferation through a CB2-dependent mechanism in Jurkat cells, with a mild pro-apoptotic activity, as proved by a significant increase in both hypodiploid peak percentage and cleaved PARP-1 levels. We confirmed that JT11 acts via CB2 by showing a substantial reduction of phospho-ERK1/2 levels in CB2 siRNA-transfected cells compared to negative control. Finally, in line with literature data, these results opened the way to the anti-inflammatory effect of JT11, which proved to modulate the inflammatory intracellular signaling, as shown by the significant reduction of LPS-induced ERK1/2 and p65-NF-kB phosphorylation and pro-inflammatory cytokines release in human PBMCs.

CONCLUSIONS

CB2 exogenous ligands could enhance the immunoregulatory activity of ECS. Our results encourage further research on the effects of JT11 on in vitro and in vivo models and suggest that therapeutic strategies aimed at selectively modulating CB2 signaling, free of CB1-related side effects, could be promising for the treatment of various chronic inflammatory diseases.

P081

MIRNAS AS A HALLMARK OF HEALTHY AGING. CORRELATION ANALYSIS IN A SICILIAN POPULATION: FROM YOUNG TO ULTRACENTENARIANS

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BACKGROUND-AIM

Aging is a series of physiological events that are usually ineluctable. It becomes a risk and accelerator factor for many age-related diseases. In turn, senescence is the biological aging of cells, caused by excessive intracellular or extracellular stress or damage. In particular, senescence of the endothelial cells (ECs) impairs vascular functions, enhancing the aging of tissues and organs. Several stimuli, including inflammatory cytokines, can speed their senescence. Recent studies have revealed that human aging can be characterized by a profile of circulating miRNAs, predictive of biological age, and that can be used as biomarker of risk for inflammageing. Recently, senescent ECs have also emerged as a possible source of circulating miRNAs.
In this research, it was studied a panel of four miRNAs: mir-146a-5p, mir-126-3p, mir-21-5p, and mir-181a, involved in several pathways related to inflammation, EC senescence, and carcinogenesis in order to identify possible miRNA candidates as hallmark of healthy aging.

METHODS

The circulating levels of these miRNAs were determined in 78 healthy subjects aged between 22 to 111 years. We classified the population in four age groups, i.e., young adults (≤50 y.o.), adults (51-70 y.o.), older adults (71-99 y.o.), ultracentenarians (≥100 y.o.). Contextually, extracellular mir-146-5p, mir-126-3p, mir-21-5p, and mir-181a levels were measured in human endothelial cells (HUVECs) cultures, undergoing senescence, under normo/oxidative conditions.

RESULTS

We found that the levels of the four miRNAs, in vivo and in vitro, progressively increase with age, with the exception of ultracentenarians that showed levels comparable to those measured in young adult individuals. In particular, mir-146a-5p shows significant differences between the older adult and the young adult groups, and between the older adults and the ultracentenarians. Moreover, we observed that oxidative stress induced in vitro different variation of extracellular miRNAs levels, depending on senescence status.

CONCLUSIONS

All levels of circulating miRNA analyzed in our cohort suggest that the longevity phenotype is characterized by a relative low inflammatory status as compared to the older adults and a possible less degree of aging of ECs.

P082

DOWNREGULATION OF INTESTINAL INFLAMMATORY SIGNALING PATHWAYS BY DIETARY FERULIC ACID AND ITS METABOLITES

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BACKGROUND-AIM

The dysregulation of intracellular signals which lead to the translocation of NF-κB is distinctive of intestinal inflammatory diseases (IBD). Different stimuli such as cytokines and bacterial lipopolysaccharide (LPS) promote permeability dysfunctions through activation of the immune system and inflammatory process. Intestinal inflammation induced by these noxious molecules is characterized by an aberrant activation PI3K/Akt and the MAPKs pathways, all leading to the expression of inflammatory enzymes such as iNOS and COX-2. It has been shown that these same pathways are sensitive to the action of bioactive compounds derived from the diet and of their phase I/ II metabolites, which may concentrate in the intestine and exert an important role in the prevention of inflammatory response.

METHODS

We evaluated the efficacy of dietary ferulic acid (FA), together with its main phase I/II metabolites, against the proinflammatory effects induced by LPS in intestinal cells, as well as the mechanisms underlying their protective action. The compounds were tested at physiological relevant concentrations to counteract LPS-induced overexpression of proinflammatory enzymes related to the activation of NF-kB and involving Akt and MAPK, as well as the expression of the transcription factor Nrf2.

RESULTS

LPS-induced overexpression of iNOS and the consequent hyperproduction of nitric oxide and cGMP were limited by FA, its derivatives isoferulic acid and dihydroferulic acid, and their glucuronidated and sulfated metabolites, which acted upstream by limiting the activation of MAPK p38 and ERK1/2 and of Akt kinase, thus decreasing NF-kB translocation into the nucleus. Furthermore, the compounds were found to promote the expression of Nrf2, which may have contributed to the downregulation of NF-kB activity.

CONCLUSIONS

This study pointed out the effects of the principal FA metabolites against intestinal inflammation acting on different signalling routes. The results confirm that the metabolic conversion of dietary FA into its metabolites does not undermine the bioactivity of the free form, but on the contrary originates compounds equally capable of preserving intestinal integrity against pro-inflammatory agents, thus contributing to hamper or limit the progression of intestinal inflammation and related diseases.

P083

IMMUNOPHENOTYPIC CHARACTERIZATION OF SICILIAN SEMI AND SUPERCENTENARIAN.

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BACKGROUND-AIM

Immunosenescence constitutes a major indirect cause of morbidity and mortality in older people. To gain insight into its mechanisms, we have recently analysed (Ligotti et al., Clin Exp Immunol. 2021) immune phenotypes in a cohort of the Sicilian population from young to the oldest living Italian supercentenarian (111 years old). The supercentenarian showed a unique immunophenotypic signature as regards the relative percentages of her T cell subsets, with CD4+ and CD8+ T cell percentages and CD4+ Naïve T cell values in line with those recorded for the octogenarian subjects. It appeared that the supercentenarian has a Naïve "younger" T cell profile, suggesting that the characteristic of the immune system constitutes the longevity secret of these special subjects.
Aims: To confirm and extend the previous studies performing further immunophenotypical analysis in ultracentenarians and controls.

METHODS

Analysis of T cells, their senescent and activated/exhausted status, $\gamma\delta$ T cells, B cells and NK performed in centenarian, semi-, and super-centenarians and controls.

RESULTS

Preliminary results seem to confirm the previous results, especially in gender differences. For example, we observed a significant reduction in Naïve and a significant increase in TEMRA CD8 T cells in females but not in males, although two ultracentenarians show Naïve CD8 T and TEMRA profiles comparable to young subjects, with Naïve CD8 T cell percentage notably high in the male semi-supercentenarian. Also, most of the female semi- and supercentenarian show the highest values of activated CD8+PD1+ T cells, a possible symptom of chronic activation of the immune system, whereas the male supercentenarian, the oldest living Italian man, shows the lowest. However, it should be noted that our females ultracentenarias largely outnumber male ones, as usually women live longer than men.

CONCLUSIONS

The present data confirm and extend previous findings showing age-related Naïve T cells decrease with a concomitant increase in the percentage of TEMRA. In addition, we observed gender differences in immunophenotype not only in younger subjects but also in semi- and supercentenarians. However, an increase in sample size, especially in centenarian males, is needed to statistically confirm these findings.

P084

TARGETING ION CHANNEL AND TRANSPORTER MACROMOLECULAR HUBS: A NOVEL APPROACH TO OVERCOME THERAPY RESISTANCE IN CANCER.

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BACKGROUND-AIM

Therapeutic antibodies usually target specific biomarkers, highly expressed in the primary tumor. However, selective markers of metastatic disease, to be exploited for therapy, are generally missing. Recently, the hERG1/ β1 integrin complex was shown to form physical and functional complexes with either NHE1 or CA-IX, in colorectal cancer (CRC) and clear-cell renal carcinoma (ccRCC), respectively. Such complexes represent functional hubs to regulate intracellular and extracelluar several aspects of cancer progression, as well as to drive pro-invasive signalling pathways.

METHODS

Human colorectal (CRC) and clear cell renal carcinoma (ccRCC) cells were used to perform in vitro (co-immunoprecipitations and western blot). Tissue slides of metastatic CRC patients and ccRCC patients were used for IHC. In vivo (subcutaneous and eco-guided xenografts) analyses were also performed. Moreover, we have developed engineered antibodies with TRAIL ligand and ADC (antibody drug conjugates) against the macromolecular hubs.

RESULTS

1.We have developed a single chain bispecific antibody, in the format of a Diabody against the hERG1/β1 complex: scDb-hERG1-β1. The scDb-hERG1-β1 reduced 3D growth, lateral motility and invasiveness, as well as pAkt in vitro, as well as tumour growth and angiogenesis, and pAkt in preclinical CRC models, in vivo. 2. We have tested the antibody and its ADC derivates on CRC and ccRCC cells demonstrating the presence of such macromolecular hubs and tested them also on IHC slides as tumor biomarkers.

CONCLUSIONS

The novel immuno-therapeutic strategy we have developed, using scDb-hERG1-β1 based ADC, could be translated to clinical studies in the near future, representing an innovative approach to target tumor macromolecular hubs, with potential diagnostic and therapeutic applications.

P085

THE EFFECTS OF MAGNESIUM DEFICIENCY ON LIPIDS DEPOSITION: THE ROLE OF EDF-1 AND PPARγ IN REGULATING ENDOTHELIAL METABOLISM

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BACKGROUND-AIM

Endothelial cells (ECs), which constitute the inner surface of blood vessels, are considered the gatekepeers of vascular integrity. They perform fundamental functions, i.e. they control the traffic of molecules between the blood and the tissues, regulate blood fluidity, vascular tone, leukocyte trafficking and the immune response, which flow into the regulation of the homeostasis of the entire organism. The leading cause of atherosclerosis, a common phenomenon in different cardiovascular diseases, is a functional dysregulation of ECs which shift towards a pro-inflammatory and pro-oxidant phenotype. Evidences indicate that low magnesium (Mg) promotes the acquisition of this phenotype. Endothelial Differentiation-Related Factor 1 (EDF-1), a small intracellular protein which plays a role in controlling endothelial performance, is expressed both in the cytosol, where it is involved in the modulation of endothelial Nitric Oxide Synthase, and in the nucleus, where it acts as transcriptional co-activator for proteins required for lipid metabolism, among which Peroxisome Proliferator-Activated Receptor gamma (PPARγ) that is involved also in the modulation of cytokine production, energy metabolism and apoptosis in ECs. Since both magnesium homeostasis and EDF-1 have crucial roles in shaping ECs function, we investigated its modulation as well the deposition of lipids in human ECs cultured in different concentrations of magnesium.

METHODS

Human endothelial cells from the umbilical vein (HUVEC) were cultured in medium containing from 0.1 to 5 mM Mg for 24h. The levels of EDF-1 and PPARγ were visualized by Western blot. Reactive oxygen species (ROS) were measured by DCFDA. The alteration of fatty acids metabolism was investigated through O Red Oil staining and metabolic assays.

RESULTS

After 24h of culture in low extracellular magnesium, we observed an upregulation of both EDF-1 and PPARγ in association with ROS production and accumulation of intracellular neutral lipids.

CONCLUSIONS

Magnesium deficiency leads to the deposition of lipids by inducing EDF-1 and PPARγ; the increase in intracellular lipids might be interpreted as an adaptive response of endothelial cells to magnesium deficiency.

P086

HERG1 ION CHANNEL INTERACTS WITH β1 INTEGRIN TO MODULATE CANCER PROGRESSION IN RAFT PLASMA MEMBRANE DOMAINS.

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BACKGROUND-AIM

Pancreatic cancer (PCa) represents one of the deadliest cancer types worldwide. This rely on its intrinsic malignant behavior and high resistance to therapeutic treatments. In this scenario, the assembly of multi-protein complexes in response to different microenvironmental extracellular cues is key for orchestrating cell behavior. Ion channels exert a role in many diseases including cancer. hERG1 ion channel is known to be overexpressed in several malignances and forms macromolecular hubs along with integrins and possibly other actors, such as lipid rafts, which are part of cell traffic and participate in cell remodeling and to cell death program execution.

METHODS

To study the localization of the hERG1/β1 Integrin complex into lipid rafts, PANC-1, pancreatic ductal adenocarcinoma (PDAC) cells, were lysed and the supernatant fraction was subjected to a sucrose density gradient. To verify whether hERG1 may bind directly to gangliosides co-immunoprecipitation experiments were performed using anti-hERG1 mAb. Cells were also treated to evaluate ERK and AKT phosphorylation. Moreover, the association of hERG1/β1 Integrin with lipid rafts in HEK 293 hERG1 cells under fibronectin stimulation was also investigated. Statistical analysis was performed throughout the study.

RESULTS

Our results demonstrate that hERG1/ β1 integrin complex mediated signal transduction pathway acts through lipid rafts in human PANC1 cells. In particular, we observed that hERG1 and Integrin beta1 interact with ganglioside GM1, a paradigmatic component of these plasma membrane compartments. Of note, in the present study we showed that ERK and AKT phosphorylation was significantly prevented by previous pretreatment with MβCD. Furthermore the association of hERG1 with β1 Integrin was mainly present into lipid rafts of HEK 293 hERG1 cells only after triggering with fibronectin.

CONCLUSIONS

In conclusion, we provide evidence that signaling involving hERG1/β1 integrin complex acts through lipid rafts. These data allow us to propose the involvement of lipid rafts in a new molecular hub formed by hERG1/β1 Integrin molecules. The latter, confirmed in PDAC cells, open the path for possible targeting for therapeutic purposes.

P087

ALTERATIONS OF THE ETHER-à-GO-GO K+ CHANNEL ELECTROPHYSIOLOGY (BIOPHYSICS) UPON INTERACTION WITH BETA1 INTEGRINS.

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BACKGROUND-AIM

hERG1 channels regulate many stages of tumorigenesis. Many of tumor processes strongly depend on regulated cell adhesion to the ECM. hERG1 and the ß1 integrin receptors can form a macromolecular complex on the plasma membrane of cancer cells. We combined experimental and theoretical methods to study the relationships between the integrin-dependent hERG1 current activation and the formation of the macromolecular complex between the channel and the β1 integrin.

METHODS

We studied the dynamics of hERG1/β1 complex formation and hERG1 current modulation upon integrin activation by cell adhesion to ECM proteins, using HEK 293 cells transfected with the hERG1 encoding cDNA (HEK-hERG1 cells) as a model. Cells were collected from preparatory cultures and seeded onto Fibronectin -coated dishes in serum-free medium for different times. Cells seeded onto BSA were taken as controls (not integrin activated cells). We monitored the time courses of cell adhesion, of hERG1/β1 complex formation (witnessed by the amount of hERG1 protein which co-immunoprecipitates with the β1 subunit of integrin receptors), of the maximal hERG1 current (IhERG1) and resting potential (Vrest) values, up to 300 min.

RESULTS

hERG1/ß1 complex progressively increased from the time of cell seeding up to 90 min, to decrease rapidly thereafter, similar to these observed at time zero. The maximal of IhERG increased between zero and 90 min. Vrest progressively hyperpolarized slowly and progressively returning to its initial values afterwards. Cells seeded onto BSA did not show any significant variations of IhERG or Vrest and there was not the formation of hERG1/β1 complex. hERG1 protein had the maximal expression on the plasma membrane at 90 min and before and after this time point hERG1 colocalized with endosomal Rab5 protein.

CONCLUSIONS

The kinetics of IhERG1 increase was specular to the trend of Vrest hyperpolarization. No significant variations of the activation and inactivation features of hERG1 currents were detected. Integrin-dependent cell adhesion triggers the formation of a hERG1/β1 complex. All these parameters reach their maximum values at 90 min, to decline thereafter because the hERG1 protein had the maximal expression on the membrane.

P088

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF CBX2 IN COLORECTAL CANCER

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BACKGROUND-AIM

Colorectal cancer (CRC) is the second leading cause of cancer mortality, and its incidence is gradually increasing in developing countries. A better understanding of CRC biology is associated with significant advances in molecular technologies, which have led to the identification of several epigenetic alterations involved in colorectal carcinogenesis. The proteins of the Polycomb group (PcG) promote gene repression through epigenetic modifications of the chromatin structure. CBX proteins regulate the activity of PRC2 with PRC1 serving as critical regulators of PcG-mediating activity. Several proteins of the PcG are deregulated in the CRC and their dysfunction is responsible for the proliferation, inhibition of apoptosis and the increase in the population of cancer stem cells. Genomic and transcriptomic studies have shown that CBX2 is very expressed in colorectal cancer compared to non-tumor models, highlighting its potential oncogenic role. We aim to to characterize the role of (epi) deregulation of CBX2 and to find an innovative approach for CRC therapy.

METHODS

Western Blot analysis, proliferation assays, viral transduction/transfection, TMA analysis, RT-PCR, FACs analysis

RESULTS

Preliminary data showed an up-regulation of CBX2 expression in the most advanced stages of CRC samples. CBX2 expression levels were assessed by immunohistochemistry assays on a tissue microarray of a cohort of CRC samples versus the non-tumor counterpart. Following CBX2 silencing in selected cell lines, the expression of K-RAS, the most known mutated gene in CRC, resulted downregulated. CBX2 silencing also had an important impact on tumor cell proliferation and migration, suggesting its promising role for effective and innovative diagnosis.

CONCLUSIONS

The epigenetic mechanisms, including DNA methylation, post-translational histone modifications, nucleosome remodelling, contribute to the regulation of gene expression and determine cell and tissue identity. Moreover, the de-regulation of these mechanisms contributes together with genetic alterations to tumorigenesis and progression. Since the increase in CBX2 expression has been correlated with lower survival of CRC patients, influencing tumor proliferation and progression, it could represent a novel tumoral predictive marker of CRC.

P089

POTENTIAL ROLE OF CXCL-8 PRODUCED BY ENDOTHELIAL CELLS IN THE ESTABLISHMENT OF LEISHMANIA INFECTION

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BACKGROUND-AIM

Leishmania promastigotes are inoculated into the skin of the human hosts by the bite of phlebotomine sandflies. Endothelial cells are one of the first cells type encountered by parasites, but little is known on their role in the recruitment of phagocytic cells and in the establishment of the infection. In the first steps of infection, neutrophils are rapidly recruited to the site of promastigotes deposition and phagocytize Leishmania promastigotes, which elude the killing mechanisms of the host cells, survive and infect other phagocytic cells.

METHODS

Human microvascular endothelial cells (HMEC-1) were co-coltured with promastigotes. Morphological changes and phagocytosis were determined by Giemsa staining and microscopic observation. The production of CXCL8 and CCL5 by HMEC-1 was evaluated by ELISA. Migration of leukocytes isolated from fresh peripheral blood human lymphocytes (PBL) was evaluated using the transwell migration assay.

RESULTS

After co-incubation with HMEC-1, Leishmania promastigotes showed significant morphological changes and loss of infectivity. Moreover, promastigotes of different Leishmania species stimulated the production of CXCL8 by HMEC-1 in a dose- and TLR4-dependent manner. In a transwell migration system, the conditioned media from Leishmania-stimulated HMEC-1 cells attracted after 2 hours leukocytes, mostly neutrophils, in higher number compared to supernatants from unstimulated HMEC-1 cells. After 24 hours, a higher percentage of monocytes was detected in conditioned media of unstimulated HMEC-1 cells, whereas neutrophils still predominated in conditioned medium from Leishmania-stimulated cells. The same supernatants did not contain CCL5, a chemokine recruiting T cells and monocytes; on the contrary, inhibition of the production of CCL5 induced by TNF-α was observed.

CONCLUSIONS

These data suggest that the interaction of Leishmania promastigotes with endothelial cells leads to the production of CXCL-8, which contribute to the establishment of Leishmania infection, through the recruitment of neutrophils to the site where promastigotes are deposited by sandflies.

P090

EXPLOITATION OF THE GUT-LIVER AXIS TO PREVENT LIVER PATHOLOGY VIA TRYPTOPHAN CATABOLISM

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BACKGROUND-AIM

Primary sclerosing cholangitis (PSC) is a long-term liver disease characterized by inflammation and the destruction of the biliary tree that leads to fibrosis or end-stage complications, such as liver cirrhosis. To date the pathogenesis of human PSC remains unknown, but it is thought to be multifactorial, influenced by genetic predisposition and immune-mediated processes. Intestinal barrier dysfunction has been implicated in the pathogenesis of PSC. According to the "leaky gut" hypothesis, gut inflammation alters the permeability of the intestinal mucosa, leading to the translocation of gut-derived products into the enterohepatic circulation with consequent hepatic inflammation. It has been shown that indoles metabolites produced by intestinal bacteria are able to control liver disease manifestations in diverse disease models. Indole-3-carboxaldehyde (3-IAld) is a microbial-derived product working at the interface between the host and the microbiota and is able to promote mucosal immune homeostasis in a variety of preclinical settings, but its potential application in PSC has never been explored.

METHODS

Herein, we resorted to a murine model of PSC based on the administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and we evaluated the effect of 3-IAld-loaded enteric microparticles (3-IAld-MP) formulated for localized delivery in the gut.

RESULTS

We found that a local hepatic activity of 3-IAld-MP alleviates liver inflammation and fibrosis by inhibiting the production of TGF-ß and IL-9 by modulating chronic inflammation in the liver. Moreover, 3-IAld-MP treatment activates the aryl hydrocarbon receptor-IL-22 axis in the gut and increases the expression of ZO-1 and Ki-67 favoring the restoration of the gut barrier function. Of interest, 3-IAld-MP significantly decreases the abundance of pro-inflammatory Enterobacteria, such as E. coli, while increases the abundance of L. reuteri that is associated with protection from bile duct ligation-induced liver injury and fibrosis.

CONCLUSIONS

This study provides evidence of the beneficial effect microbial metabolites, such as 3-IAld, may have liver pathology by acting through the "gut-liver axis" involving the local microbiota.

P091

POST-TRANSLATIONAL MODIFICATIONS OF PROTEINS IN EXTRACELLULAR MICROVESICLES IN PERIPHERAL BLOOD IN RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Extracellular vesicles (EV) are lipid bound vesicles secreted by cells into the extracellular space in both physiological and pathological conditions. They play a role as intracellular "communication mediators" due to their ability to transfer cell components that can regulate various biological processes. An increase in EV levels has been described in autoimmune diseases, which may be responsible for immune responses to self-antigens. Recent studies showed that post-translationally modified (PTMs) proteins may be present in EVs in patients with Rheumatoid Arthritis (AR). In this study, we focused on the identification of citrullinated and carbamylated proteins in EVs in patients with active AR.

METHODS

Peripheral blood samples from naïve patients with early-active AR and healthy donors (HD) were centrifuged to obtain purified EV. Samples were divided into three aliquots, for NanoSight, flow cytometry and western blot analysis. “Subtypes”of microvesicles (MV) from different cell populations were identified by specific antibodies (anti-CD62 for platelets, anti-CD45 for leukocytes and anti-CD31 for endothelial cells); PTMs were analyzed using antibodies to citrullinated and carbamylated proteins.

RESULTS

Our results demonstrated, by NanoSight analysis, a significant increase (about 3 times) of number of microvesicles in AR patients compared to healthy donors. Moreover, flow cytometry analysis proved an increase of citrullinated ($p=0.03$) and carbamylated ($p=0.8$) proteins, in AR-MV compared to HD-MV. Western blot analysis allowed to identify the cytoskeletal protein vimentin, the cytoplasmatic glycolytic enzyme alpha-enolase1 and the collagen II type, as the main citrullinated and carbamylated proteins carried by MV. Furthermore, a significant correlation was found between carbamylated ($p=0.04$) and citrullinated proteins ($p=0.003$) of MV and DAS28 in RA patients.

CONCLUSIONS

PTMs of proteins, such as citrullination and carbamylation, carried by EVs in AR patients, suggest that these modified proteins are recognized by the immune system together with MHC molecules, inducing autoantibodies production. These observations introduce a new pathogenetic mechanism of the disease, thus providing a useful tool for monitoring and diagnosing patients with RA.

P092

ULTRASOUND-BASED METHOD FOR IDENTIFICATION OF NOVEL MICRORNA BIOMARKERS IN PROSTATE CANCER

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BACKGROUND-AIM

Detection of circulating microRNA (miRNA)-based biomarkers represents an innovative, non-invasive method for early detection of cancer. However, low concentration of miRNAs released in body fluids, especially in early-stage disease, and difficult localization of the tumour site limited their clinical use as effective cancer biomarkers.

METHODS

To evaluate if ultrasound treatment could amplify the release of extracellular cancer biomarkers, we treated a panel of prostate cancer (PCa) cell lines with an innovative ultrasound-based prototype and profiled the release of miRNAs in the extracellular space, with the aim of identifying novel miRNA-based biomarkers that could be used for PCa diagnosis and monitoring of tumour evolution.

RESULTS

We provided evidence that US-mediated sonoporation amplify the release of miRNAs from both androgen-dependent (AD) and –independent (AI) PCa cells. We identified four PCa-related miRNAs, whose levels in LNCaP and DU145 supernatants were significantly increased following ultrasound treatment: mir-629-5p, mir-374-5p, mir-194-5p and let-7d-5p. We further analysed a publicly available dataset of PCa, showing that the serum expression of these novel identified miRNAs was upregulated in PCa patients compared to controls, thus confirming their clinical relevance.

CONCLUSIONS

Our findings highlight the potential of using ultrasound to identify novel free-cell miRNAs released from cancer cells, with the aim of developing new biomarkers with diagnostic and predictive value.

P093

THE HISTONE METHYLTRANSFERASE EZH2 PREVENTS THE ONCOSUPPRESSIVE ROLE OF NOTCH SIGNALING

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BACKGROUND-AIM

Notch signaling deregulation can promote or counteract tumorigenesis depending on cellular context. Up to date, several Notch inhibitors are under clinical evaluation for the treatment of Notch-addicted tumors, whereas fewer efforts have been spent to develop Notch-activating approaches as potential therapeutic strategies for those malignancies in which Notch acts as tumor-suppressor.

Recent studies have revealed a promising potential of epigenetic-based therapy for Notch signaling modulation. Indeed, methylation status of H3K27 orchestrates transcription of NOTCH and its target genes, and the balance between its tri-methylated and demethylated state is regulated by the histone methyltransferase EZH2 and the histone demethylase JMJD3. JMJD3 inhibition was successfully used to counteract oncogenic Notch signaling in T-cell acute lymphoblastic leukemia and colon cancer, whereas the potential of a specular inhibition of EZH2 for reactivation of oncosuppressive Notch transcriptional program has not been elucidated yet.

METHODS

HL-60 and U937, HeLa, SiHa, cell cultures; In vitro pharmacological treatment; RNA interference; RT-qPCR; Nuclear-cytosol extraction; Western blotting; Trypan blue exclusion assays.

RESULTS

Since JMJD3 sustains Notch oncogenic program in several tumors, we hypothesized that, in a specular manner, EZH2 could represses Notch signaling in acute myeloid leukemia (AML) and cervical cancer (CC), in which Notch might have an oncosuppressive function. Confirming our hypothesis, EZH2 inhibition reactivated Notch signaling and reduced cell proliferation in HL-60 and U937 AML cells and HeLa and SiHa CC cells. Of note, the treatment resulted in increased expression of the differentiating factor CEBPα in AML and the negative cell cycle regulators p27 and p21 in CC. Moreover, we demonstrated that Notch inhibition partially rescued the anti-cancer effect of EZH2 inhibition in SiHa and HL-60 cells, but not in HeLa and U937 suggesting the existence of additional regulatory circuits.

CONCLUSIONS

Altogether, our findings indicate that EZH2 oncogenic function is partially related to the repression of Notch oncosuppressive program and suggest inhibition of EZH2 as a therapeutic strategy based on Notch signaling reactivation in appropriate cancer contexts.

P094

OPTIMIZING THERAPEUTIC OUTCOMES OF IMMUNE CHECKPOINT BLOCKADE BY A MICROBIAL TRYPTOPHAN METABOLITE

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BACKGROUND-AIM

Despite the great success, the therapeutic benefits of immune checkpoint inhibitors (ICI) in cancer immunotherapy are limited by various resistance mechanisms or ICI-associated toxic effects including gastrointestinal toxicity. Thus, novel therapeutic strategies that provide manageable side effects to existing ICI would enhance and expand their therapeutic efficacy. Due to its proven role in cancer development and immune regulation, gut microbiome has gained increasing expectation as a potential armamentarium to optimize immunotherapy with ICI. We have assessed whether microbial metabolites working at the interface between microbes and the host immune system may optimize ICI therapy.

METHODS

We have tested indole-3-carboxaldehyde (3-IAld), a microbial tryptophan catabolite known to contribute to epithelial barrier function and immune homeostasis in the gut via the aryl hydrocarbon receptor (AhR), in a murine model of ICI-induced colitis. Epithelial barrier integrity, inflammation and changes in gut microbiome composition and function were analyzed. AhR, IDO1, IL-10 and IL-22 knockout mice were used to investigate the mechanism of 3-IAld activity. The function of the microbiome changes induced by 3-IAld were evaluated upon fecal microbiome transplantation (FMT). Finally, a murine melanoma model was used to assess the effect of 3-IAld treatment on the anti-tumor activity of anti-CTLA-4.

RESULTS

Upon administration to mice with ICI-induced colitis, 3-IAld protected mice from intestinal damage via a dual action on both the host and the microbes. Indeed, paralleling the activation of the host AhR/IL-22-dependent pathway, 3-IAld also affected the composition and function of the microbiota such that FMT from 3-IAld-treated mice protected against ICI-induced colitis with the contribution of butyrate-producing bacteria. Importantly, while preventing intestinal damage, 3-IAld did not impair the anti-tumor activity of anti-CTLA-4 in murine melanoma.

CONCLUSIONS

This study provides a proof-of-concept demonstration that moving past bacterial phylogeny and focusing on bacterial metabolome may lead to a new class of discrete molecules that working at the interface between microbes and the host immune system may optimize ICI therapy.

P095

BIOLOGICAL SCREENING OF A STERICALLY-HINDERED CURCUMINOID LIBRARY IN T-ALL CELL LINES

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BACKGROUND-AIM

Deregulated Notch signaling is linked to the pathogenesis and resistance to common therapeutic agents of multiple tumors, including T-cell acute lymphoblastic leukemia (T-ALL), thus indicating its targeting among the most promising anti-cancer strategies. Nevertheless, most of the current Notch-inhibiting approaches showed disappointing efficacy and poor safety profile in clinical studies, encouraging the development of novel Notch inhibitors as candidate anti-leukemic drugs. The natural polyphenol Curcumin showed promising anti-tumor properties by exerting antiproliferative and proapoptotic effects via Notch signaling inhibition in experimental models of different solid tumors, thus suggesting further investigations on the anti-Notch and anti-leukemic potential of curcumin-based compounds in Notch-driven T-ALL. Of note, curcumin's clinical application is restricted due to its poor absorption and reduced stability and cellular uptake. Therefore, extensive efforts have been devoted to developing novel curcumin-derived compounds with improved pharmacological properties.

METHODS

T-ALL cell lines (Jurkat, P12-ICHIKAWA, and TALL-1) were treated with curcumin and 17 its derivatives at different concentration and times. Anti-viability effects were evaluated with Trypan Blue exclusion assay. Molecular effects were evaluated by Western Blot and RT-PCR analysis.

RESULTS

The in vitro screening of 17 curcumin derivatives, with substituted aromatic ring or linker region, against different Notch-dependent T-ALL cells allowed us to select a novel curcumin-based compound, namely Cu15, endowed with potentiated anti-viability and Notch-inhibitory capacity compared with curcumin. Additionally, we observed that the Cu15-mediated Notch inhibition correlated with DNA damage signatures and increased levels of the pro-apoptotic cleaved form of poly ADP- ribose polymerase (PARP). Finally, the constitutive exogenous expression of the active domain of the Notch1 receptor partially rescued the anti-viability effects of Cu15 suggesting that it acts at least in part via Notch inhibition.

CONCLUSIONS

Overall, our findings suggest the Cu15 molecule as a novel Notch inhibiting agent with anti-proliferative properties in T-ALL worthy of further investigation and development.

P096

GENDER-SPECIFIC TRANSCRIPTOMIC IMMUNE PROFILES IN BRCA-ASSOCIATED BREAST CANCERS

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BACKGROUND-AIM

Compared with breast cancer (BC) in women, BC in men is rare and less investigated. Clinical management of male BC (MBC) patients is informed by female BC research; however, increasing evidence suggests gender-specific differences. Inherited BRCA1 and BRCA2 (BRCA) mutations predispose to BC in both sexes and characterize a subgroup of tumors with a peculiar phenotype.
Here, we aimed to perform a transcriptome-based immune profiling in male BCs and to compare immune profiles between male and female BCs, in relation to BRCA mutation status.

METHODS

A well-characterized series of 59 invasive MBCs, including 21 BRCA-associated MBCs, were analyzed. Whole transcriptome data were obtained by RNA-sequencing using Illumina technology. Immune profiles were evaluated using CIBERSORT and ESTIMATE. Data on 1,100 female BCs (FBCs), including 20 BRCA-associated FBCs, from The Cancer Genome Atlas were used for comparison.

RESULTS

A strong positive correlation was observed between transcriptome-based tools and immunohistochemistry in evaluating Tumor Infiltrating Lymphocytes (TILs) (Adjusted R^2=0.73; p<0.0001). MBCs with higher immune scores showed higher expression of PDL-1 (p=0.0005), higher tumor mutational burden (p=0.04), a more frequent lymph nodal involvement (p=0.03) and a trend toward worse overall survival (Log-rank p=0.18). Overall, MBCs showed lower immune scores compared with FBCs (p=0.02).
In analysis restricted to BRCA-associated BCs, comparable immune score levels were observed in male and female BCs. Notably, BRCA-associated MBCs had lower PD-1 expression levels compared with female BRCA-associated BCs (p<0.0001).
BRCA-associated MBCs and FBCs showed statistically significant (p<0.05) differences in the fractions of several immune cells: M1 Macrophages, Gamma-Delta T cells, Plasma cells, resting Dendritic cells and Neutrophils, were more abundant in FBCs; resting CD4+ memory T cells, Monocytes, activated NK cells and Eosinophils, were more abundant in MBCs.

CONCLUSIONS

Our results showed that transcriptome-based evaluation of BC immune profiles may be a valuable approach for dissecting biologically and clinically relevant gender-specific immune features of breast tumors.
Study supported by AIRC (IG21389) to LO.

P097

A POSSIBLE LINK BETWEEN GUT MICROBIOME COMPOSITION AND CARDIOVASCULAR COMORBIDITIES IN PSORIATIC PATIENTS

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BACKGROUND-AIM

Psoriasis is a chronic inflammatory and immune-mediated disease characterized by cutaneous and systemic manifestations, that can be associated with several comorbidities, including cardiovascular disease (CVD).
Gut dysbiosis has been suggested to play a role in the development and maintaining of both psoriasis and CVD.
The aim of this study was to investigate a possible link between gut microbiome and the occurrence of CVD in psoriatic patients by comparing the gut microbiome composition in psoriatic patients with and without CVD.

METHODS

The series consisted of 28 psoriatic patients, including 17 with and 11 without CVD. The main clinical data, including therapeutic treatment, were collected for all patients. In particular, nine patients (five with and four without CVD) were in treatment with anti-psoriatic biologic drugs.
For each patient, fecal bacterial DNA was extracted and analyzed by 16s rRNA sequencing on Illumina platform. Bioinformatics and statistical analyses were performed to compare the gut microbial composition between the two groups of patients.

RESULTS

A significantly higher abundance of bacteria known to promote systemic inflammation (Barnesiellaceae, p=1.05E-12 and Phascolarctobacterium, p=2.65E-15) was observed in psoriatic patients with CVD, compared with those without CVD.
Notably, psoriatic patients with CVD undergoing treatment with anti-psoriatic biologic drugs showed lower abundance of Barnesiellaceae (p=7.79E-11), compared with untreated patients, with abundance levels comparable to those of psoriatic patients without CVD.

CONCLUSIONS

This study highlights a specific gut microbiome composition in psoriatic patients with and without CVD and suggests a positive impact of anti-psoriatic biologic drugs on the gut microbiome. Our findings indicate that strategies aimed at restoring the gut microbiome symbiosis might potentially contribute to suppress the systemic inflammation, representing useful approaches in the management of both psoriasis and CVD.

P098

EVALUATION OF RAS AND BRAF MUTATIONAL STATUS IN LIQUID BIOPSY TO MONITOR DISEASE PROGRESSION OF METASTATIC COLORECTAL CANCER.

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BACKGROUND-AIM

Metastatic colorectal cancer is characterized by a high frequency of KRAS mutations that are the main determinants of the failure of anti-EGFR-based therapy. Moreover, BRAF mutations at the valine 600 residue are also responsible of poor prognosis (Van Cutsem E et al., 2011) due to the limited response to EGFR inhibitors.
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). RAS and BRAF status are currently determined in tissue samples but performing multiple biopsies should be avoided, therefore it was proposed the use of liquid biopsy as a surrogate of tissue sample.

METHODS

BRAF, K- and NRAS status were determined on tissue by Next Generation Sequencing (NGS) at the hospitals of Pistoia and Pescia. 8ml of peripheral blood were collected and 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

The concordance between BEAMing and NGS revealed an excellent agreement for both KRAS and NRAS. The results obtained comparing NGS and Real Time Quantitative PCR (Idylla) revealed a good agreement for NRAS and BRAF. The agreement for KRAS was lower probably due to the different sensitivity of the techniques.
In addition, for some patients it was possible to monitor the mutational profile during the course of treatment with samples taken 4, 8 and 12 weeks after the commencement of the therapy. Although the values of MAF (Mutant Allele Fraction) among the various patients are variable, but in the majority of patients the presence or absence of KRAS mutations is maintained during the course of therapy, as well as the presence or absence of BRAF mutations.

CONCLUSIONS

Our data suggest that RAS and BRAF mutations should be performed in both tissue and plasma at the baseline and on plasma during follow-up to better monitor disease progression and define the best treatment options for each patient.

P099

PERIPROSTATIC ADIPOSE TISSUE PROMOTE PROSTATE CANCER CELL MIGRATION BY PARACRINE TGFβ UPREGULATION OF CTGF

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BACKGROUND-AIM

Epidemiologic studies indicated that obesity is an important adverse prognostic factor for several types of cancer, including prostate cancer (PCa). Prostate gland is largely surrounded by periprostatic adipose tissue (PPAT). Recent studies showed that adipocyte-released factors can affect the early stage of PCa enhancing the spread of cancer cells outside the prostate gland. Little is known about the molecular mechanism linking PPAT and PCa aggressiveness.
In this study we investigated the ability of adipocytes-released factor to enhance PCa cell migration.

METHODS

Adipose-derived mesenchymal stem cells (Ad-MSCs) were obtained from biopsy of PPAT. Ad-MSCs were differentiated in mature adipocytes and then serum starved to obtain conditioned media (Ad-CM). Two prostate androgen-independent cancer cell lines, DU145 and PC3, were used to assess the effect of AD-CM on cell migration, performing scratch assay. Levels of TGFß were evaluated in Ad-CM using ELISA assay. CTGF and pSMAD expression were analyzed by Western blot analysis.

RESULTS

Culture with Ad-CM 48h with Ad-CM from PPAT enhanced cell migration in both DU145 and PC3 cell lines. Interestingly, the pretreatment of DU145 and PC3 with SB 431542, a well-known TGFß receptor inhibitor, counteracts the ability of Ad-CM to induce migration. Using ELISA assay, we demonstrated that PPAT mature adipocytes released TGFß. Furthermore, Ad-CM treatment induced the upregulation of CTGF and incubation with SB 431542 plus Ad-CM reduced this effect.

CONCLUSIONS

Our data suggest that adipocyte-released TGFß may influence migration inducing the upregulation of CTGF providing a better understanding of the relevance of PPAT in PCa microenvironment for cancer cells dissemination.
These findings reveal the potential of the identification of new prognostic biomarkers and pharmacological targets for the treatment of PCa.

P100

THE ROLE OF HISTIDINE-RICH GLYCOPROTEIN (HRG) IN NASH-RELATED EXPERIMENTAL LIVER CARCINOGENESIS

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BACKGROUND-AIM

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

METHODS

The role of HRG, was investigated by morphological, cellular and molecular biology approaches in: a) HRG knock-out mice (HRG-/- mice) undergoing a NASH-related protocol of hepatocarcinogenesis; b) NAFLD patients carrying HCC; c) THP1 and HSC/MF cells treated with purified HRG.

RESULTS

Data obtained showed that HRG: a) is expressed in HCC nodules and released in plasma samples from NAFLD-related patients, b) exerts an M1-type pro-inflammatory action against monocytes / macrophages, c) is able to stimulate some of the pro-fibrogenic responses in human myofibroblasts. Moreover, following the treatment with the DEN/CDAA protocol, HRG-/- mice showed a significant decrease in the volume and number of liver tumors as compared to wild-type mice. These effects were not associated with a modulation of cell proliferation process and may be attributable to a reduction in the angiogenic response together with an increase in the apoptotic process.

CONCLUSIONS

These results indicate that the release of HRG by hepatocytes has a critical role in the progression of experimental liver carcinogenesis in a dietary NAFLD/NASH-related environment.

P101

METABOLIC REPROGRAMMING IN OSIMERTINIB-RESISTANT NSCLC CELL LINES

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BACKGROUND-AIM

Osimertinib is the third-generation, mutant selective EGFR tyrosine kinase inhibitor approved for the first-line treatment in NSCLC (Non Small Cell Lung Cancer) patients. Despite the efficacy of osimertinib in first- and second-line treatments, patients inevitably develop acquired resistance. The mechanisms of resistance can be EGFR-dependent or independent and another strategy by which cancer cells may escape from the selective pressure of drug and become resistant is metabolic reprogramming.

METHODS

Glucose, glutamine and lipid metabolism were investigated in a panel of NSCLC cell lines with different mechanisms of resistance. Metabolic parameters were measured by Seahorse XFp analyser. Cell lipidome was profiled by UHPLC coupled to high-resolution mass spectrometry with ion-mobility separation.

RESULTS

Considering the role of the EGFR signaling pathway in the modulation of glucose metabolism, we investigated the effects of osimertinib on energy metabolism in sensitive (OS) and resistant (OR) NSCLC cells. Osimertinib decreased glucose uptake and consumption in OS cells. The OR cells with an EGFR-dependent mechanism retained the ability to utilize glucose exhibiting a glycolytic phenotype. By contrast in OR cells with EGFR-independent mechanisms glucose uptake was decreased. Moreover, cell proliferation was slightly affected by glucose deprivation suggesting a metabolic reprogramming towards the utilization of alternative fuel sources. The OR cells were characterized by an increased intracellular content of lipids in comparison with the parental cells. Furthermore, the lipidomic analysis showed an increase in (poli)glicosylceramides when compared to OS cells. Interestingly, inhibition of the glucosylceramide synthase (GCS) by PDMP reduced cell survival by inducing cell death in OR cells.

CONCLUSIONS

The role of energy cell metabolism in acquired resistance remains undefined. The identification of changes in metabolic pathways may clarify their role in the acquisition of drug resistance unveiling new approaches for overcoming resistance in the clinical practice. Our data suggest the potential involvement of GCS in osimertinib-resistance, and the possibility to overcome resistance by inhibiting the biosynthesis of glycosylated ceramides.

P102

HOPS/TMUB1: CHARACTERISING A NEW MODULATOR IN CELLULAR SENESCENCE

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BACKGROUND-AIM

Two of the main hallmarks of cancer are resistance to cell death and the ability to replicate cell immortally. In the past 20 years, apoptosis and aging, as tumour escape mechanisms have been well characterized, but there is still much to be discovered. A new role of a ubiquitin-like modifier, HOPS (Hepatocyte Odd Number Protein Shuttle) has been characterized. HOPS is able to interact with and control p19 half-life and localization and recently, it has been discovered that HOPS is involved in p53 transcription-independent pathway, increasing its cytoplasmic protein stability, thereby enhancing its ability to undergo apoptosis after genotoxic stress. Considering the importance of HOPS in apoptosis p53-mediated and its involvement in controlling p19 stability, cellular senescence has been investigated.

METHODS

Wild-type (WT) and hops knockout (KO) Mouse Embryonic Fibroblasts (MEFs) were prepared from C57BL/6 mice and hops KO C57BL/6 mice. mRNA extracted has been evaluated by qPCR. The total proteins collected from MEFs were analysed via Western Blot. β-galactosidase (β-gal) activity has been assessed by both a histochemical assay and a fluorescence detection.

RESULTS

Comparing WT and hops KO MEFs, it was observed that the mRNA expression levels associated with the two main aging markers, p16 and p19ARF, were reduced in hops lack. In addition, comparing WT and KO senescent cells, it has been showed that the protein expression of the two senescence markers modify their expression patterns. In particular, although p16 and p19 levels gradually increased in WT MEFs, relatively low protein amounts and defective activation were detected in KO MEFs over time. Further evidence of the role of HOPS was found in protein stability assays. In the KO cells, p16 and p19 are rapidly degraded and the half-life is shortened compared with WT cells. Evaluation of β-gal activity, a marker of aging, showed a delayed expression in KO MEFs.

CONCLUSIONS

This study identified HOPS as a regulator of tumor escape by promoting its activation throughout control of important senescence partners. These results indicate for the first time HOPS as a potential candidate at the crossroads of the tumourigenic response, inducing apoptosis or senescence, and may be selectively reactivated and up-regulated in tumour cells.

P103

HOPS/TMUB1 MEDIATES P53-DEPENDENT STRESS RESPONSE

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BACKGROUND-AIM

The tumor suppressor p53 represents the most supervised protein in cancer biology, playing a pivotal role in protecting cells from neoplastic transformation. In response to stress stimuli, p53 accumulates in the nucleus and guards the genome by coordinating a variety of DNA-damage-response mechanisms involving cell cycle arrest or apoptosis. In recent studies we showed that following etoposide double-strand breaks, the HOPS/TMUB1 protein binds and stabilizes p53, contributing to its mitochondrial apoptotic program. With the relevance of p53 in maintaining genome stability and the emerging role of HOPS in affecting p53 cytoplasmic functions, we investigated HOPS capacity to participate in multiple p53-mediated DNA-repair mechanisms.

METHODS

We used a combination of genetic, bioinformatics and biochemical approaches in order to provide novel information on the global functioning of HOPS in mediating p53 functions. In Hops knock out MEFs and Hela cells, generated using Crispr/Cas9 tech, we monitored the transcriptional and non-transcriptional p53 activities in both murine and human systems following stress stimuli, such as UV-irradiation and H_2O_2 treatments.

RESULTS

Following genotoxic stimuli, we observed a HOPS-dependent modulation of the apoptotic p53 response at mitochondria, with a significant decrease in Bax and caspase 3 activation. Interesting, IF analysis of Hops knock out cells showed a reduction in p53 localization in the cytoplasm, matching the drop of p53 fraction at mitochondria. HOPS depletion cause a diminution of percentage of apoptotic cells in response to stress stimuli, confirming the involvement of HOPS in affecting p53 apoptotic response. We also analyzed HOPS impact on p53 transcriptional activity and we reported a de-regulation of the major p53-dependent apoptotic genes, besides a functional interaction assessed by luciferase assays, deeply connecting HOPS to the p53 apoptotic tasks.

CONCLUSIONS

Given the many ways through which p53 guards the genome, it remains a major challenge to dissect its multiple functions, especially regarding the interplay with HOPS in promoting apoptosis and limiting tumorigenesis. Further studies will enrich the understanding of the physiological roles of the HOPS/p53 axis especially in genome evolution and translational medicine.

P104

HUMAN ENDOGENOUS RETROVIRUS DRIVES INSL4 EXPRESSION IN LUNG CANCER

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BACKGROUND-AIM

Non-Small Cell Lung Cancer (NSCLC) is the leading cause of cancer related death in both genders. Our studies allowed us to classify INSL4, a placenta specific gene, as an oncogene. INSL4 is overexpressed in several NSCLC cell lines and in 5% of NSCLC patients in which correlates with worst OS. INSL4 is physiologically expressed only in placenta. Here we investigate the oncogenic mechanisms of INSL4 in NSCLC.

METHODS

Bioinformatic analyses were performed using Zenbu 3.0, Cancer Cell Line Encyclopedia, Nascent Transcription Repository database. Non-canonical transcript was detected by RT-PCR.

RESULTS

Bioinformatic analysis of INSL4 gene was carried out, showing, upstream of the gene, the presence of a Human Endogenous Retrovirus (HERV) with probable activity on INSL4 expression. HERVs in the genome are epigenetically silenced by DNA methylation, therefore we proceeded by analyzing the methylome of 92 NSCLC cell lines, this analysis showed that the expression levels of INSL4 were highly correlated with upstream HERV methylation status, suggesting the presence of alternative promoters contained in the HERV, INSL4 overexpression would be therefore strongly influenced by epigenetic factors. Through the RNA-Seq Analysis approach in A549 it is possible to find reads signals consistent with the Transcription Starting Site (TSS) for the INSL4 canonical transcript, but also anomalous reads in the LTR of the HERV indicating a non-canonical TSS upstream of INSL4 and a functional reactivation of the HERV. In this, we also identified a putative binding site for ARID3A, a central transcription factor in the placenta. The presence of a TSS within the LTR sequence led us to hypothesize about the existence of a non-canonical and cancer-specific INSL4 transcript. Through RT-PCR analysis on A549 cells it was possible to amplify a transcript fragment that begins inside HERV and continues inside INSL4, suggesting the existence of cancer-specific transcript.

CONCLUSIONS

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

P105

SERUM POLYCLONAL FREE LIGHT CHAINS: A POSSIBLE MARKERS OF IMMUNE ACTIVATION IN PSORIASIS?

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BACKGROUND-AIM

Polyclonal free light chains (FLCs) of the immunoglobulins include κ and λ chains and represent a sensitive marker of the activation and/or dysfunction of the immune system. They have been widely investigated in onco-hematology, but evidences show that they increase in chronic inflammation or autoimmune diseases too. The aim of this study was to investigate the role of FLCs as markers of immune activation in the management of psoriatic patients on treatment with biologics.

METHODS

The overall study population included 30 patients affected by mild-to-severe psoriasis with either ongoing biological treatment or without any current systemic therapy and 10 healthy controls. FLCs were determined by quantitative nephelometric assay while antinuclear antibodies (ANA) through immunofluorescence.

RESULTS

Psoriatic patients showed significant increased levels of κ and λ FLCs compared to healthy controls. Interestingly, κ and λ FLCs values were significantly increased only in psoriatic patients with ongoing biological treatment and in particular in responder subjects. Furthermore, correlation of both κ and λ FLCs with duration of therapy showed significant results. Patients with FLC levels above the normality range and under biological treatment for more than 12 months showed higher odds to be ANA+ respect to patients with FLC levels above the normality range but under biological treatment for less than 12 months.

CONCLUSIONS

Increased FLCs levels would seem to act as markers of immune reactivation in psoriatic patients on treatment with biologic agents suggesting that the determination of FLCs could have clinical relevance in psoriasis clinical management.

P106

AN AGONIST OF THE CXCR4 RECEPTOR IS THERAPEUTIC FOR THE NEUROPARALYSIS INDUCED BY BUNGARUS SNAKES ENVENOMING

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BACKGROUND-AIM

We report the therapeutic properties of a small molecule agonist of the CXCR4 receptor on the recovery from the peripheral flaccid neuroparalysis caused by Bungarus snakebites. Snake envenoming is a neglected disease that, each year, causes > 100,000 deaths mainly in tropical and sub-tropical areas of the world. Recently we discovered that the degeneration of the motor axon terminal is accompanied by the expression of the CXCR4 receptor on the neuronal plasma membrane and that a small molecule CXCR4 agonist, dubbed NUCC390, promotes the recovery of function of the degenerated NMJ.

METHODS

We employed in vivo analysis on envenomed mice, mimicking the intoxication observed in patients. We performed local intoxication with both purified bungarus toxin and their total venom. We followed up nerve recovery from a morphological and functional point of view by immunofluorescence at the neuromuscolar junction and by electrophyiological analysis.

RESULTS

The venoms of Bungarus snakes cause a rapid and complete degeneration of the motor axon terminal. This is accompanied by the expression of the CXCR4 receptor. The recovery of function of the NMJ can be quantitatively assessed by electrophysiology performed on single muscle fibers or on the entire muscle. NUCC390 accelerates the recovery of NMJ function after the neuroparalysis caused by Bungarus venoms or by β-BTX. The positive effect of NUCC-390 on the single muscle fiber is paralleled by the one assessed on the entire muscle.
A major symptom of envenomation by Bungarus snakes is the respiratory deficit that may be so extensive as to cause death. We tested the effect of NUCC390 on the recovery of the respiratory function in envenomed mice by ventilation assay. Bungarus venoms decrease both the frequency and extent of ventilation with changes in shape and dimension of each event. NUCC390 treated animals already after 24 h showed an increase in peak area, and this effect amplified with the progress of time. At 96 h, NUCC390 treated mice showed a complete recovery in lung ventilation, a result reached by control animals only after one week.

CONCLUSIONS

The data presented here qualify NUCC390 a novel potential therapeutic to accelerate nerve structural and functional recovery after the neurodegeneration and paralysis caused by Bungarus snake poisoning.

P107

OMENTIN-1, A POTENTIAL BIOMARKER FOR IRON DEFICIENCY IN PATIENTS ON HEMODIALYSIS

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BACKGROUND-AIM

Omentin-1 is a 40 kDa anti-inflammatory adipokine released by visceral adipose tissue and negatively correlated with obesity, insulin resistance, and coronary artery disease. In addition, this adipokine is involved in the regulation of iron metabolism by binding lactoferrin.
Hemodialysis (HD) patients are often characterized by iron deficiency, due to abnormal iron absorption, external blood loss, hemodialysis treatment itself, and inflammation. In the last case, functional iron deficiency can be masked by high ferritin levels.
The aim of this pilot study was to evaluate serum levels of omentin-1 in a small HD population, in order to determine its possible relationship with iron status.

METHODS

85 chronic HD patients and 15 healthy subjects matched with HD patients by gender and BMI, were enrolled in this multicentric study. Following routine biochemical and clinical parameters, serum omentin-1 was measured by a Sandwich-ELISA kit (Human Intelectin-1/Omentin, Novus Biologicals-Canada) before a single mid-week HD session and at 1h, 2h and 3h after dialysis start.

RESULTS

Serum omentin-1 levels were statistically higher in HD patients than in matched healthy controls [784 (447.6-1519) vs. 228 (67.9-366.1) ng/mL; p=0.03]. Omentin-1 levels were dramatically reduced after the first hour of HD (reduction ratio: 45±5%) and tended to get back to baseline after the third hour (p=0.04). Correlation analyses showed serum omentin-1 levels to be directly associated with serum iron (R=0.380; p=0.03), ferritin (R=0.843; p<0.0001), and transferrin saturation (TSAT) (R=0.661; p<0.0001).
ROC analysis showed a remarkable capacity of serum omentin-1 to discriminate HD patients with iron deficiency (TSAT $\leq$20% and serum ferritin $\leq$200 μg/L) (AUC = 0.907), with a sensitivity of 86.3% and a specificity of 89.2%.

CONCLUSIONS

This pilot study demonstrates that HD patients have increased levels of omentin-1. As this protein is involved in the maintenance of iron equilibrium, omentin-1 might be proposed as a new tool in the assessment of iron deficiency and, prospectively, in the management of iron therapy in HD patients. Additional studies are, however, needed to confirm, and possibly, to generalize these findings.

P108

MELATONIN PROMOTES REGENERATION OF INJURED MOTOR AXONS

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BACKGROUND-AIM

Melatonin is an ancient molecule synthesized by the pineal gland and by several extrapineal tissues. A variety of activities have been attributed to this hormone in different physiological and pathological contexts, but little is know about its role in peripheral nervous system regeneration.

METHODS

In this work, we have exploited two different types of injury to test the capability of melatonin to stimulate regeneration of motor axons: i) the acute and reversible presynaptic damage induced by the spider neurotoxin α-Latrotoxin, and ii) the compression/transection of the sciatic nerve. We used electrophysiological techniques such as Compound muscle action potentials (CMAPs) and Evoked junction potentials (EJPs) recordings to measure nerve conduction and to assess neuromuscular junction functionality after melatonin treatment. We also evaluated axon regeneration by imaging, with antibodies specific for presynaptic markers such as Neurofilaments and Syntaxin.

RESULTS

We found that in both cases melatonin treatment accelerates the process of nerve regeneration. This pro-regenerative action is at least in part due to a sustained activation of the ERK1/2 pathway, and it is mediated by the interaction with melatonin receptors type 1, as incubation with selective receptor antagonists, slows down neurotransmission rescue upon peripheral damage.

CONCLUSIONS

These findings reveal a receptor-mediated, pro-regenerative action of melatonin which holds important clinical implications, as it posits melatonin as a safe candidate molecule for the treatment of several peripheral neurodegenerative conditions.

P109

UNRAVELING THE INTERPLAY BETWEEN HEDGEHOG-GLI AND ERK5 PATHWAYS IN MELANOMA

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BACKGROUND-AIM

Malignant melanoma is the deadliest skin cancer, with a poor prognosis in advanced stages. We recently showed that the Mitogen-Activated Protein Kinase ERK5 plays a relevant role in melanoma growth. The Hedgehog signalling pathway (HH-GLI) controls cell proliferation and differentiation during embryonic development, and its altered activation has been described in several types of cancer, including melanoma. Of note, no data are available in literature for a possible interplay between HH-GLI and ERK5.

METHODS

BRAFV600E melanoma cells A375 and SK-Mel-5 were silenced for ERK5 using two different ERK5 targeting shRNAs (shERK5-62 and shERK5-75) and a non-targeting shRNA (shNT), as a negative control.

RESULTS

To deepen how ERK5 controls the growth of melanoma cells, we performed transcriptomic experiments after ERK5 knock-down (KD) in melanoma cells. By meta-analysis, we found that GLI3 gene, one of the components of HH-GLI pathway, was one of the most upregulated genes. qRT-PCR analysis confirmed that GLI3 was upregulated in both A375 and SK-Mel-5 ERK5-KD cells. TNMplot in silico data of melanoma tumor samples (T=253 patients) and normal skin samples (N=174 patients), revealed that GLI3 expression was much lower in tumoral samples than in normal samples comparing. Intriguingly, melanoma patients with increased GLI3 expression showed a significant increased overall survival. GLI3 exists in two forms, a full length weak activator (GLI3-FL) and a truncated repressor (GLI3-R). In order to identify which one is increased upon ERK5 inhibition, we performed western blotting experiments in ERK5-KD cells. Preliminary results indicate an increase of GLI3-FL in both cell lines. However, the fact that the expression of GLI1, a downstream target of the pathway, is reduced in both ERK5-KD cells seems to suggest a shutdown of HH-GLI pathway, rather than an activation, pointing to the existence of a regulatory mechanisms elicited upon ERK5 inhibition on the HH-GLI pathway.

CONCLUSIONS

The results of the present study shed light on new downstream effectors of ERK5 signalling, improving the knowledge necessary to develop new therapeutic strategies for the treatment of melanoma and, possibly, other neoplasms where ERK5 and HH-GLI pathways are involved.

P110

EVALUATION OF ANTIINFLAMMATORY EFFECTS OF OLEUROPEIN AND HYDROXYTYROSOL IN PBMC TO TEST THEIR EFFICACY AS NEW ADJUVANTS FOR VACCINES.

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BACKGROUND-AIM

The anti-inflammatory effects of phenols contained in extra virgin olive oil (EVOO), and in particular of oleuropein (OLE) and hydroxytyrosol (HT), have been described several times in in vivo and ex vivo models.
The objective of this project, which is part of a much more ambitious and complete European project (ISOLDA, Improved Vaccination Strategies for Older Adults, funded by H2020-EU.3.1.3.), is to evaluate the anti-inflammatory effects of EVOO compounds (HT and OLE) on PBMCs, obtained from blood samples of healthy young and elderly volunteers.

METHODS

The samples used were obtained thanks to the voluntary participation of healthy subjects in the project. PBMCs were extracted from blood samples and placed on a 96-well Costar plate. The plates were placed in a CO2 incubator, at 37 °C, with a pH of 7.4, for 2 hours and then treated with phytochemicals. The concentrations used to treat the cells were: 1/5/10 µMol, for both OLE and HT, added to the complete wells and treated.
After 2 successive hours of incubation, LPS (1µg / ml) was added to the individual wells to stimulate the inflammatory process.
The production of the levels of pro-inflammatory cytokines, IL-1β and TNF-α, released by the PBMCs, was carried out by means of an ELISA assay.

RESULTS

The data highlighted the anti-inflammatory effect of HT and OLE on the treated cells.

CONCLUSIONS

These preliminary data show that these extracts of EVOO exert an anti-inflammatory effect on primary cultures of PBMCs after stimulation with bacterial LPS and consequently could be promising compounds for the formulation of new anti-influenza vaccines.

P111

PROTEIN SIGNATURES FOR NON-SMALL CELL LUNG CARCINOMA (NSCLC) EARLY DIAGNOSIS AND MONITORING

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BACKGROUND-AIM

Non-small-cell lung carcinomas (NSCLC) is the most common type of lung cancer and it has a poor prognosis, because overall survival after 5 years is 20–25% for all stages. Thus, it is extremely important to increase the survival rate in the early stages NSCLC by focusing on novel screening tests of cancer identifying specific biomarkers expression associated with a more accurate tumor staging and patient prognosis.
In this study, we focused our attention on quantitative proteomics of three heavily glycosylated serum proteins: AMBP, α2 macroglobulin, and SERPINA1.

METHODS

Human whole blood was obtained by venipuncture of patients with non-small cell lung cancer aged 30–70 years at the Department of Cardio-Respiratory Disease, Thoracic Surgery Unit, University of Campania "Luigi Vanvitelli", according to the institutional bioethics code.
Proteins from 25 μl of serum obtained by patients (Thoracic Surgery Unit) were precipitated with 1 ml of 5% trichloroacetic acid in acetone and resuspended in denaturant buffer composed of 6 mol.L−1 urea in 50 mmol.L−1 NH4HCO3 then dithiothreitol (DTT) 500 mmol.L−1 were added to reach the final 10 mol.L−1 DTT concentration . Finally, the samples were diluted with 50 mmol.L−1 NH4HCO3 to reach the final 0.6 mol.L−1 urea concentration and trypsin was added to ensure a minimum enzyme-to-substrate ratio of 1:50. Enzymatic digestion was carried out overnight at 37°C and then stopped by adding formic acid at a final concentration of 0.1%. Samples were dried and stored a −80°C. Samples were reconstituted with 100 μl of 0.1% formic acid (HCOOH) aqueous solution for subsequent HPLC-MS/MS analysis.

RESULTS

We analyzed serum samples from 20 patients in early and advanced stages, and 10 healthy donors to quantificate AMBP, α2 macroglobulin, and SERPINA1 through the MRM analysis that have shown to be markers of cancer development and progression. Our results showed a decrease in SERPINA1 expression in patients affected by advanced cancer stage whereas bikunine increases its expression in those patients. The expression of α microglobulin instead remains unaffected.

CONCLUSIONS

In conclusion, we believe that proteins like α2 macroglobulin, αmicroglobulin/bikunin, and SERPINA1 could be useful biomarkers for early detection of lung cancer and in monitoring its evolution.

P112

POTENTIAL CYTO-GENOTOXICITY OF RUSSIAN CHRYSOTILE FIBRES: IN VITRO RISK ASSESSMENT

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BACKGROUND-AIM

Asbestos is a global human health hazards related to malignant diseases, represented by a wide group of mineral fibers (chrysotile, amphibole and erionite) with different chemical and physical characters. Thanks to bio durability, resistance to high temperature, insulating properties and cheapness, it has provided an extensive use in industry and in residential areas. Several studies report a variety of human health damages (pleural plaques, asbestosis, lung cancer, mesothelioma) caused by asbestos airborne particulate matter inhaled. Despite of the IARC report, that classifies the asbestos fibers as potential carcinogens for human, asbestos are still used in several countries as China, Russia, India. Several chrysotile mines are still active in Russia, even if the effective potential toxicity of Russian chrysotile has not been investigated.

METHODS

The aim of our study was therefore to investigate the effects exerted in vitro of two different Russian Chrysotile (Chry-Ru) fibers whose lengths are <5μm and >5μm. Experiments were performed in humans mesothelial (MeT5A) and epithelial (A549) cell lines, to assay: a) cytotoxicity by light microscope morphological investigations and MTT assay to test cell viability, b) genotoxic effects by Comet assay to detect DNA damage in single cells at time points, according to specific parameters: tail length (TL); tail moment (TM) and tail intensity (TI).

RESULTS

Results obtained showed that Chry-Ru fibres >5 μm, compared to the shorter fibres, induced in both cell lines: higher grade of cell vacuolization, attesting an early alteration of normal cell physiological state; a significant decreased cell viability at 6, 24 and 48hrs ($p<0.01$); early DNA damage since 6h, according to Comet parameters.

CONCLUSIONS

Preliminary results suggest potential cyto-genotoxic effects exerted by Chry-Ru longer fibers according to literature [Boulanger G et al, Environ Health. 2014]. Deeper investigations are required to elucidate the molecular mechanisms involved.

P113

EFFECT OF CYCLIC(RGDYK)-GEMCITABINE MOLECULAR CONJUGATES ON SARCOMATOID AND EPITHELIOID MURINE MESOTHELIOMA CELL LINES

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BACKGROUND-AIM

In the last years, targeted anticancer therapy, known as "targeted drug-delivery", has been widely improved toward the development of new compounds with high specificity and selectivity. In this view, the RGD-class integrin receptors, which recognize the tripeptide sequence (arginine-glycine-aspartic acid) have been used as a molecular target, because of their massive expression on cancer cells. Cilengitide is a cyclic pentapeptide derived from the RGD sequence. Unfortunately, clinical trials revealed that Cilengitide alone did not improve the outcomes of patients receiving chemo- and radiotherapy but Cilengitide itself represents a milestone in targeted anticancer therapy. Thus, a number of researchers focused their efforts on the development of anticancer agents derived from the conjugation of Paclitaxel, or Doxorubicin with Cilengitide or RGD-mimetic peptides. In this work, we focused on the effects of newly synthesized compounds obtained by the conjugation of gemcitabine with the cyclic mimetic RKDyK.

METHODS

Malignant pleural mesothelioma (MPM) is a very aggressive disease, with a very low life expectancy and a lack of functional therapeutic approaches. We evaluated the anti-proliferative and pro-apoptotic activities of two newly synthesized compounds (TC113 and TC116) on AB1 and AB22 murine mesothelioma cells that resemble features of human sarcomatoid and epithelioid MPM phenotypes, respectively. MPM cells were grown in 2D and 3D culture conditions. Co-cultures with L929 murine fibroblasts were prepared, in the presence or in the absence of TGFβ, to exacerbate the role of the stromal component in the progression and aggressiveness of mesothelioma.

RESULTS

We found that TC113, the most stable conjugate, was able to significantly reduce the proliferation of mesothelioma cells. This effect was reduced when the cells were exposed to TGF-β, which is known to confer resistance to drugs. These results were confirmed using a 3D model and highlighted that TC113 was more effective than gemcitabine in reducing the growth of co-cultured spheroids enriched in cancer cells.

CONCLUSIONS

These preliminary results will be improved by in vivo investigation, in order to evaluate the potential role of the conjugates to selectively target MPM cancer cells and reduce the side effects on healthy tissue.

P114

SENSITIVE DETECTION OF SARS-COV-2: A NOVEL IN-CELL ELISA ASSAY

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BACKGROUND-AIM

Coronaviruses (CoV) are a family of related microorganisms that infect the respiratory tract in humans. In the global coronavirus epidemic, many SARS-CoV-2 infections are asymptomatic or have only mild symptoms but can still transmit the virus to others. The detection of viral RNA using RT-PCR in respiratory specimens was recognized as the gold standard for the diagnosis of a SARS-CoV-2 infection. However, the main limitation of this technique is that it takes several hours to generate results; in addition, specialized instruments and expertise are required. In the severe SARS-CoV-2 pandemic, it is necessary to identify devices for making rapid diagnoses to reduce the spread of the disease.
The aim of this study was to provide a qualitative, rapid, sensitive, and specific method for the diagnosis of a SARS-CoV-2 infection based on the recognition of specific antigens of SARS-CoV-2 that can also be performed in environments outside the laboratory, i.e., "patient side," with an immediate chemocolorimetric response or with a digital reader.

METHODS

The kit box was organized in rows of nine wells/stations containing buffers, lysis systems, and detection systems, as well as lyophilized antibodies, all of which were stable at room temperature (ELISA Test). Furthermore, the kit was equipped with tools for the collection of biological material and support for the PVDF strip, which was armed with the primary specific antibodies, spike and nucleocapsid.

RESULTS

This method was based on the assessment of SARS-CoV-2 virus particles through the determination of the spike and nucleocapsid proteins using an ELISA test. The device allows you to quickly perform a test that directly evaluates the presence of the virus in the biological sample, in about 30 min. It offers the advantage that the result of the antigen–antibody reaction can be directly visible to the naked eye without the need to be carried out in a laboratory, without analytical instrumentation, and above all, without the need to transport the samples.

CONCLUSIONS

In conclusion, the high sensitivity of the method guarantees an early diagnosis, even in those subjects with borderline SARS-CoV-2 positivity. Its signal cut-off point makes it unique among diagnostic systems; in fact, our test has a particularly low sensitivity limit (5 pg/μL).

P115

PURE LIGHT SCATTERING ANALYSIS ON CIRCULATING TUMOUR CELLS BIOPHYSICAL PROFILE: NEW TRENDS IN PRECISION MEDICINE FOR NON-SMALL CELL LUNG CANCER (NSCLC) DIAGNOSIS .

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BACKGROUND-AIM

Nowadays cancer diagnosis and treatment mostly uses solid biopsy from primary or secondary tumours. This procedure retains several disadvantages both for the patient, which is object of a real invasive surgery technique and for the hospital since this procedure is very expensive and time consuming. Such difficulties are particularly of in-terest when dealing with tumours having difficult surgical access and a high mortality rate, such as non-small cell lung cancer (NSCLC).
The aim of this study was to present a new morphological measurement approach for the detection of vital CTCs from pleural washing in individual non-small cell lung can-cer (NSCLC) patients.

METHODS

After a diagnosis of pulmonary malignancy, pleural washing was collected from 9 NSCLC patients. The collected samples were processed with a density gradient separation process. Light scattering analysis was performed on single cell.

RESULTS

Results of such analysis was used to get cells biophysical pattern and, later on, as basis for Machine Learning (ML) on un-known samples. Morphological single cell analysis followed by ML show a predictive picture for a NSCLC patient, screening that it is possible to distinguish circulating tumour cells (CTCs) from other cells. Moreover, we find that the proposed measurement approach was fast, reliable, label-free, identifying and count CTCs in a biological fluid.

CONCLUSIONS

In conclusions our findings demonstrate that CTCs Biophysical Profile by Pure Light Scattering in NSCLC could be used as a promising diagnostic candidate in NSCLC patients.

P116

HUMAN EXPOSURE TO HEAVY METAL POLLUTANTS AND MALE FERTILIY: DEMOGRAPHIC AND SOCIO-ECONOMIC MONITORING

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BACKGROUND-AIM

Environmental exposure to toxic pollutants, have been indeed demonstrated to negatively affect male fertility in humans. Based on pathophysiological effects, trace metals can be divided into two sub-groups: the first consists of 15 microelements essential for life (arsenic, cobalt, chromium, copper, fluorine, iron, iodine, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc) as implicated in important metabolic processes and the second includes toxic micro-elements, such as cadmium (Cd), mercury (Hg), chromium (Cr) and lead (Pb) for living organisms, even at low concentrations. Numerous evidence of the negative role of heavy metals on reproduction has been provided by experimental studies in different in vitro and animal models to contribute to general qualitative deductions on agent toxicity and to increase accuracy in quantitative risk assessments.
The aim of this study was to describe the biological effects of heavy metal pollution on male reproductive system and underline socio-economic impact induced by the damage caused by pollutants.

METHODS

Peripheral venous blood and spermatic samples were collected to determine the concentration of the following heavy metals: lead (Pb), cadmium (Cd), mercury (Hg), vanadium (V) nickel (Ni) and Arsenic (As), e polychlorinated biphenyls (PCBs).
Bio-Functional Sperm Parameters were studied in association with mitochondrial membrane potential (MMP) and sperm motility, to evaluate sperm apoptosis by flow cytometry.

RESULTS

The evaluation of serum and spermatic heavy metal presence and the bio-functional sperm analysis allowed us to identify lipid peroxidation (LP) and mitochondrial superoxide levels measurements as rational oxidative stress indices.A direct proportionality between the concentration of heavy metals and the alterations of the mitochondrial membranes and infertility was shown.

CONCLUSIONS

In conclusion, heavy metal exposure male pollution contribute to serious consequences male infertility including a demographic decrease together with consequent economic effects.

P117

COMBINATORY ANTICANCER ACTIVITY OF DL922-947 ADENOVIRUS AND G-QUADRUPLEX BINDERS AGAINST BREAST CANCER

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BACKGROUND-AIM

G-quadruplex (G4) are nucleic secondary structures characterized by G-tetrads. G4 motif stabilization induces DNA damage and cancer cell death; therefore, G4-targeting small molecules are the focus of clinical investigation. DNA destabilization induced by G4 ligands might potentiate the anticancer activity of agents targeting DNA or inhibiting its repair, a mechanism observed in oncolytic viruses (OVs). In this study, we addressed a new anticancer approach combining G4 ligands, BRACO-19 (B19), pyridostatin (PDS) and the oncolytic adenovirus dl922-947 in breast cancer cells.

METHODS

We used breast cancer MDA-MB-231 and MCF-7 cells to evaluate the anticancer effects of G4 ligands, dl922-947 and/or their combination. Cell cytotoxicity was determined by sulforhodamine B assay. Cell cycle progression, G4 structure formation and viral entry were evaluated by flow cytometry. dl922-947 DNA amplification was quantified by RT-qPCR. SA-β-gal staining was used to assess cell senescence evaluated by light microscope and blue cell count.

RESULTS

G4 ligands and dl922-947 efficiently inhibited cell viability. G4 binders induced G4 motif stabilization distributed in the S and G2/M phases, in particular in MCF-7 cells G4 motifs were also observed in the subG0/G1 following B19 treatment. The combination G4 binders-dl922-947 increased viral entry and replication in both the cell lines. Enhanced cytotoxicity was observed using the combination dl922-947-PDS with respect to the single agents in MDA-MB-231 cells. These effects correlated with increase of the subG0/G1 phase of the cell cycle. The agents used singularly or in combination enhanced cell senescence. Noteworthy, we observed that dl922-947 induced G4 structure formation and the combination with PDS potentiated this effect.

CONCLUSIONS

We provide the first evidence of a new mechanism of action of the adenovirus dl922-947 able to induce senescence and G4 motifs in breast cancer cells. We suggest a novel strategy based on the use of G4 binders/virotherapy combination to enhance anti-cancer activity. Indeed, OVs and G4 ligands are already singularly employed in clinical settings, thus their combination may be envisioned for translational studies and might represent a novel therapeutic anticancer approach.

P118

T-CELL MEDIATED IMMUNE RESPONSE TO SARS-COV-2 VACCINATION IN RHEUMATIC PATIENTS TREATED WITH B-CELLS-TARGETED THERAPIES

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BACKGROUND-AIM

The recent availability of SARS-CoV-2 vaccines has raised concerns about their efficacy in patients on immunomodulatory treatments. It has been proposed that B-cells-targeted therapies may interfere with the development of a protective immune response upon vaccination. The aim of the study was to evaluate T-cell mediated response to SARS-CoV-2 vaccination in patients with rheumatic diseases under treatment with Rituximab (RTX, mAb anti-CD20) and Belimumab (BLM, mAb anti-B-Lymphocytes stimulator BLyS).

METHODS

Patients under RTX (n=11) or BLM (n=16) treatment were vaccinated with either mRNA-1273 or BNT162b2. Total antibodies (IgG+IgM+IgA) against the receptor-binding domain (RBD) of the Spike glycoprotein were quantified using the Elecsys Anti-SARS-CoV-2 S ECLIA. SARS-CoV-2-specific T-cell response was evaluated by measuring cytokines released upon SARS-CoV-2 Spike 1 antigen stimulation (IGRA test, Euroimmun). Pre-pandemic (n=11) and vaccinated (n=13) healthy individuals were enrolled as controls.

RESULTS

All healthy controls developed humoral and cellular immune response to SARS-CoV-2 vaccination. B-cell depletion after RTX treatment impairs seroconversion, while T-cell response was detected in the majority of the patients (73%). In contrast, the treatment with BLM at the time of vaccination appears to preserve both humoral and cellular responses to SARS-CoV-2 vaccination. Very few patients (3/11 RTX and 1/16 BLM) resulted negative for both Spike 1 and mitogen stimulation, which was used as unspecific T cell stimulation.

CONCLUSIONS

Recent studies highlighted that early and robust T-cell response has been associated with mild/asymptomatic COVID-19 infection, even in the absence of antibodies. The development of a T-cell mediated immune response in patients undergoing B-cell targeted therapies is encouraging, although it is crucial to investigate whether it can provide long term protection against SARS-CoV-2 infection and re-infection.

P119

STUDY OF IL-6 AND ITS SOLUBLE RECEPTOR COMPLEX IN COVID-19 PATIENTS: POSSIBLE APPLICATION FOR PROGNOSTIC PURPOSE

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BACKGROUND-AIM

Starting from December 2019, a novel coronavirus, was identified as the responsible of a severe disease involving, in particular, lungs. This virus has been called SARS-CoV-2 and COVID-19 is the disease caused by it.
A lot of studies are indicating that mortality, following severe disease, is correlated with hyper-inflammation after viral infection, due to a cytokine storm. One of the major orchestrator is inteleukin-6 (IL-6) which plays an important role in the cytokine release syndrome and IL-6 elevated level is an hallmark of inflammation which has been detected in serum of patients with severe COVID-19 acute respiratory distress (ARDS).
The aim of this work was to demonstrate that analyzing blood inflammation indicators such as IL-6 and its receptor complex and TNF-α , it is possible to identify critically COVID-19 patients and to predict their poor prognosis.

METHODS

This study was conducted on N=23 critically patients confirmed COVID-19 and admitted in Intensive Care Unit (ICU), from March 10 to April 30 2020, following severe respiratory failure.
Serum levels of IL-6, sIL-6R, sgp130 and TNF-α were determined using CLIA and ELISA as immunoassay.

RESULTS

The data obtained have shown that not all patients had significantly high levels of sIL-6R, agonist receptor, compared to the controls. Based on these data, patients were stratified in two groups (1 and 2). In both groups IL-6 and TNF-α serum levels were significantly increased compared to the controls, but serum levels of sgp-130 antagonist receptor are significantly decreased in both groups of patients.

CONCLUSIONS

Probably this dysregulation in blood inflammation mediators is responsible of a different prognosis and pharmacological response in severe COVID-19 patients. Further studies are necessary to investigate the role of IL-6 and its receptor complex during COVID-19 severe disease and the involvement of other cytokine as well as IL-17 and IL-22.

P120

NEW OLIVE PRODUCTS ENHANCE THE EFFICACY OF GLEEVEC ON CML CELLS

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BACKGROUND-AIM

Chronic myeloid leukemia (CML) is characterized by the expression of the BCR/Abl oncoprotein, which sustains autonomous survival and growth of myeloid cells. Despite the marked efficacy of the targeted therapy of CML based on Imatinib-mesylate (IM; Gleevec) in inducing remission of disease, several patients undergo relapse. This is likely due, at least in part of the cases, to the persistence in bone marrow of BCR/Abl-negative cell subsets which are obviously resistant to Gleevec. Recently, the broad properties and low toxicity of olive oil and leaf compounds emerged as capable to potentiate of the efficacy of anti-cancer treatments. Here, we used an olive leaf extract enriched in oleuropein (OLE) and oleocanthal (OC), a compound derived from extra virgin olive oil, to verify whether these nutraceutical products could promote the maintenance of BCR/Abl oncoprotein, thus potentiating the efficacy of Gleevec on CML cells.

METHODS

We tested by MTT assay the effects of OLE and OC on cell viability in cultures of K562 and KCL22, stabilized CML cell lines; then we tested by flow cytometry the efficacy of the co-treatment with Gleevec and OLE or OC using the AnnexinV-PI assay. We also determined by western blotting the effects of treatment on BCR/Abl protein. Finally, to determine whether OLE and OC may also potentiate the inhibitory effect of Gleevec on glycolysis, we analyzed HIF-1α expression by western blotting and the metabolic profile using a Seahorse analyzer in K562 cells.

RESULTS

We found that OLE and, especially, OC were effective in increasing BCR/Abl protein expression in CML cells, a finding which prompted us to test these compounds in combination with Gleevec. In the presence of OLE or OC, the efficacy of Gleevec was increased significantly. A preliminary test on cells isolated from a CML patient confirmed that OC potentiates the cytotoxicity of Gleevec. Furthermore, OC exerted an important inhibitory effect on HIF-1α expression and glycolytic activity, which might support the enhancing effect of OC on Gleevec treatment.

CONCLUSIONS

Overall, this study opened new perspectives in view of the use of a non-toxic strategy to enhance Gleevec efficacy in CML.

P121

NATURAL COMPOUNDS AND CHEMOTHERAPY: THE EFFECT OF GENISTEIN ON CHEMORESISTANT GASTRIC CANCER CELL LINES

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BACKGROUND-AIM

Gastric Cancer (GC) is one of the most important tumors of our times. It's the fifth cancer in the whole word and the fourth cause of cancer death. GC is most frequent in Asia, especially in China and Japan but in the last decade it has increased his incidence in western countries, often combined to chemoresistance, which makes healing even more difficult.
In the last ten years, some molecules of natural origin have been studied as potential adjuvants in traditional chemotherapy. Among these, Genistein, an isoflavone found in soy, has been highlighted as pro-apoptotic and anti-proliferating agent in several tumors.
In this study we analyzed the effect of Genistein on a chemo resistant GC cell line (ACC201).

METHODS

First, we evaluated the effect of the main drugs used in GC, such as 5fluorouracil, cisplatin and paclitaxel. We set up a dose-response curve to identify the IC50 for each drug, then we started to treat the cells by administrating each drug with increasing doses for 48h, starting from a sub-IC50 dose. After six months of treatment, we evaluated the chemoresistance by MTT assays obtaining an increased IC50. Once we got the chemo-resistant cells, we studied the effect of Genistein on them. We created a dose-response curve and, we treated cells with a sub-IC50 dose, setting up proliferation curves.
We then evaluated the pro-apoptotic effect of genistein in flow cytometry through TMRE, a fluorescent probe internalized by functional mitochondria.

RESULTS

We first obtained the stabilization of resistant GC cell lines against 5Fluorouracil, Cisplatin and Paclitaxel. After genistein treatment, we observed an increase in cell death, confirmed both by cell counts and proliferation curves.
The TMRE assay evaluated the pro-apoptotic effect of Genistein. We calculated the ratio between the number of live and apoptotic cells. In control groups the values are similar for all cell lines, whereas in groups treated with Genistein there is a reduction of the ratio, particularly for 5FU resistant cells.

CONCLUSIONS

These first results suggest that genistein may play an adjuvant role in gastric cancer therapy, particularly in patients in whom traditional chemotherapy is not sufficient in counteracting tumor progression.

P122

ACID-ADAPTED MELANOMA CELLS RELEASE MIR-214-ENRICHED EXTRACELLULAR VESICLES MEDIATING MACROPHAGE-DEPENDENT TUMOR CELL EXTRAVASATION.

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BACKGROUND-AIM

Extracellular acidosis characterizes the microenvironment of most solid tumors and is now considered a hallmark of cancer, being a direct consequence of the high glycolytic metabolism of cancer cells and the reduced drainage of tumor tissues. In the last decades, much evidence highlights the role of the acidic tumor microenvironment (TME) in tumor progression. Here, we describe a new pro-tumoral mechanism sustained by acidosis, consisting of the release of miR-214-enriched extracellular vesicles (EVs) that mediate tumor cell extravasation, a key step of the metastatic cascade.

METHODS

Conditioned media of B16 melanoma cells grown under standard conditions (pH 7.4 ± 0.1) or chronic extracellular acidosis (pH 6.7 ± 0.1 >3 months) were collected and EVs isolated via the ultracentrifugation technique. EV-RNAs were isolated and miR-214 level evaluated by qPCR analysis. EVs were administered to RAW 264.7 macrophages which were then evaluated for M1/M2 polarization via cytokines and nitric oxide detection, and nuclear translocation of NF-κB. miR-214 over-expressing macrophages were also generated by a stable transfection to establish the role of miR-214 in M1/M2 polarization. EV-treated macrophage conditioned media were administered to endothelial cells to evaluate vascular permeabilization via BSA-FITC permeability assay and tumor cell transendothelial migration.

RESULTS

We observed that the EVs released by the acid-adapted melanoma cells contain a higher level of miR-214 compared to non-acidic ones. miR-214-enriched acidic-EVs promote M1 macrophage polarization as confirmed by the increased production of pro-inflammatory cytokines (IL-1ß, IL-6, TNF-α) and nitric oxide, and the nuclear translocation of NF-κB. miR-214-overexpressing macrophages were tested as well, confirming the role of miR-214 in M1 polarization. When the conditioned medium of the acidic-EV-treated M1 macrophages was administered to the endothelial cell monolayer, the increased vascular permeabilization facilitates tumor cell extravasation.

CONCLUSIONS

Here we described for the first time how the miR-214-enriched EVs, released by acid-adapted melanoma cells, induce M1 macrophage polarization and facilitate tumor cell extravasation, reinforcing the notion of the role of tumor acidosis in cancer dissemination.

P123

TERT INHIBITION IMPAIRS CELLULAR PROLIFERATION, ALTERING MYC PATHWAY, AND CAN BE EXPLOITED AS AN EFFICIENT ANTICANCER APPROACH IN VIVO

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BACKGROUND-AIM

TERT, the catalytic component of telomerase, besides its canonical role of maintaining telomeres, shows other functions promoting tumor growth/progression. We have previously demonstrated that short-term telomerase inhibition by BIBR1532 (BIBR) impairs cell proliferation, with accumulation of cells in S-phase, in vitro, in vivo and in human malignant B cells xenografted in zebrafish, without affecting telomere length. Here, we investigate the molecular mechanism(s) of how TERT inhibition impairs cell cycle progression in vitro and the possible application of TERT inhibitors in combination with antineoplastic drugs to counteract tumor growth in vivo.

METHODS

TERT positive malignant B cells were treated with BIBR or DMSO, as control, for 24 hours (h). MYC and p21 were studied at mRNA level by real-time PCR and at protein level by western blot in cytoplasmic and nuclear fractions. p21 was visualized by immunofluorescence as well. Cells were also CM-DiI labeled and injected into the yolk sac of 72h post fertilization zebrafish embryos. The embryos were then placed in medium with or without drugs [2 mM cyclophosphamide (CY) or 5 μM fludarabine (FLU)]. The number of fluorescent cells was monitored in dissociated embryos by flow cytometry at 24, 48 and 72h post treatment (hpt).

RESULTS

BIBR mediated S-phase arrest in malignant B cells was characterized by more than 50% of transcriptional and translational downregulation of MYC and an enhanced p21 expression and nuclear accumulation. Furthermore, BIBR and drugs treatment decreased xenografted cells proliferation compared to control cells in untreated embryos, with BIBR showing the higher inhibitory effect at each time point. Importantly, the combination of BIBR and drugs treatment further impaired xenografted cells proliferation, with more than four-time decrease, particularly at 48hpt for CY and 72hpt for FLU.

CONCLUSIONS

In vitro data suggest that short-term TERT inhibition by BIBR impairs cell proliferation through downregulation of MYC, thus altering its subsequent transcriptional program. Moreover, in vivo results support the use of TERT inhibitors in combination with antineoplastic drugs as an efficient anticancer approach.

P124

COVID-19 AND LOCAL INFLAMMATORY STATUS IN PREGNANT WOMEN: A SPOTLIGHT ON ADIPOSE TISSUE

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BACKGROUND-AIM

Pregnancy is characterized by an altered inflammatory profile compared to the non-pregnant state, with a finely regulated balance between pro- and anti-inflammatory cytokines. Although symptomatic COVID-19 is mostly defined by pneumonia, it has been documented that extrapulmonary systemic hyperinflammation plays a crucial role in clinical manifestations. However, the role played by local inflammation in the COVID-19 pathogenesis remains to be clarified.

METHODS

11 pregnant women with SARS-CoV-2 confirmed infections and 8 healthy pregnant women were admitted for an elective Caesarean section (35–40 weeks of gestation) at AOU Federico II of Naples. Subcutaneous adipose tissue (SAT) samples were obtained in all pregnant women from the laparotomy site. SAT biopsies were incubated in serum-free media for 24h and the SAT-conditioned media were collected and screened for the concentration of cytokines, chemokines and growth factors using a Multiplex assay.

RESULTS

A greater percentage of pregnant women with other pathologies on admission developed severe COVID-19 disease with O2 supplementation (83.3% vs 16.7%; p=0.0357). SAT-secreted molecules from COVID-19 infected pregnant women displayed an increased inflammatory profile compared to healthy pregnant. In details, both anti-inflammatory IL-1ra, IL-4 and IL-10 and pro-inflammatory molecules Eotaxin and IFN-gamma significantly increased in conditioned media obtained from SAT in COVID-19 pregnant patients.

CONCLUSIONS

In the subset of patients admitted with severe and critical COVID-19, the pregnant women displayed coexisting pathologies. Furthermore, we found increased levels of many pro-inflammatory and suppressive cytokines in SAT of COVID-19 infected pregnant women compared to healthy pregnant subjects regardless of BMI and of the presence of comorbidities. Whether these changes could affect maternal and/or foetal long-term outcomes remains to be addressed in further prospective studies.

P125

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

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BACKGROUND-AIM

Among the most aggressive and lethal types of existing tumors, pancreatic and oral cancer are counted as "big killers". 5-ALA induces the production of Protoporphyrin IX (PpIX), a natural endogenous photosensitizer in the heme biosynthesis pathway. Photodynamic therapy (PDT) is based on the reaction of the light-activated PpIX with molecular oxygen, leading to cell death due to an increased intracellular reactive oxygen species (ROS). Administration of exogenous 5-ALA improves the PpIX production by tumor cells, increasing the rates of cell death after thermal stress. Aladent (ALAD) is a medical device consisting of a heat-sensitive gel containing 5% of 5-ALA that can easly penetrate cell membranes. This study intends to test the effect of ALAD-PDT on oral (CAL-27) and pancreatic (CAPAN-2) cancer cell lines.

METHODS

Overall, CAL-27 and CAPAN-2 cell lines were incubated with ALAD in variable concentrations: 0.05% (0.23mM), 0.2% (0.92mM), 0.4% (1.84mM), 0.75% (3.45mM), 1% (4.6mM), 1.5% (6.9mM) for 2, 3, 4, 8 h. Consequently, cells were irradiated by 630 nm ±10 nm FHWM nm-wavelength Led device (TL-01). The cytotoxic effects of ALAD-PDT were measured using MTS assay. These results were compared to ALAD-untreated and non-irradiated cells. To evaluate the selective toxicity of ALAD we carried out the same treatment on normal keratinocyte cells (HaCat). Furthermore, apoptosis and cell cycle alterations were assessed by flow cytometry.

RESULTS

The MTS assay gave the highest cell mortality rate for ALAD-treated CAL-27 at 1.84mM for 8h (79.28%), while for the ALAD-treated CAPAN-2 at 0.23mM for 4 h (99.35%). Cytotoxic effect was not observed on ALAD-untreated and non-irradiated cells. On HaCat cell lines, cell viability decreased to 45,6% when treated with ALAD at 1.84mM for 4h and to 39,2% when treated with ALAD at 0.23mM at 8 h. In CAL-27 cells a significant cell apoptosis was induced after treatment with ALAD-PDT at 1.84mM for 8h. Analysis of cell cycle phase arrest revealed cell growth arrest in S/G2 in the same conditions.

CONCLUSIONS

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

P126

NGS-BASED MONITORING OF THE SPREAD OF SARS-COV-2 VARIANTS IN UDINE, ITALY.

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BACKGROUND-AIM

Since the beginning of the pandemic, Next Generation Sequencing (NGS)-based studies of the SARS-CoV-2 genome helped in the identification and tracking of novel variants of concern (VOC). In late December 2020, an increasing number of cases assigned to the VOC 202012/01 (lineage B.1.1.7, WHO-designated VOC Alpha) was registered. In this work, we report the evolution of two lineages in the province of Udine, in the north east of Italy. In particular, we tracked the onset and the diffusion of B.1.1.7 (Alpha) and B.1.621 (Mu) lineages between January and May 2021.

METHODS

All nasopharyngeal swabs resulted positive during this timeframe have been analysed by High Resolution Melting (HRM) technique. Some of them were randomly selected (N=849) to undergo NGS. Sequencing was performed with the Ion S5 GeneStudio System using the Ion 510 & Ion 520 & Ion 530 Kit-Chef and the Ion 530™ chip-kit (all ThermoFisher Scientific). Lineage assignment was confirmed by both PANGO (https://cov-lineages.org/lineages/lineage_B.1.621.html) and UShER (https://genome.ucsc.edu/cgi-bin/hgPhyloPlace) online tools. Phylogenetic analysis was performed with Molecular Evolutionary Genetics Analysis (MEGA) v.11.

RESULTS

A continuous increase in the number of samples assigned to the VOC Alpha has been evidenced, which reached the 100% prevalence in about two months. Over time, we tracked the occurrence of rare mutations in the B.1.1.7 background. Finally, we identified a cluster of B.1.621 (Mu) lineage-related samples, probably attributable to international travels as pandemic containment measures started to be mitigated.

CONCLUSIONS

This work suggests that a thorough monitoring of the SARS-CoV-2 genome by NGS is of great significance to assess lineage distribution and to contain the possible outbreak of new variants that could jeopardize all the efforts that have been made so far to resolve the emergence of the pandemic.

P127

IL-33/ST2L PATHWAY DYSREGULATION PROMOTES CARDIAC FIBROTIC REMODELING IN RAT ANIMAL MODEL OF OBESITY

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BACKGROUND-AIM

IL33/ST2L pathway exerts a fundamental protective role in cardiac tissue. In particular it modulates the release of cytokines and mediators and the activation of mast cells in response to cellular stress or damage, protecting the heart from maladaptive remodeling. In the heart IL-33 reduces fibrosis, inflammation and infarct size while increases cardiac function and survival. Interestingly, IL33/ST2L signaling is also related to fat deposition. In fact it is related to epycardial adipose tissue (EAT) thickness. On the other hand, the soluble form of ST2 receptor (sST2) is a decoy receptor, which blocks IL33 signaling. Serum sST2 levels correlated to cardiovascular disease severity representing an important clinical biomarker of heart failure.
Here we assessed IL33/ST2 pathway in a rat animal model of obesity, to evaluate the role of fat accumulation in cardiac steatosis and fibrotic remodeling driven by IL33/ST2 dysregulation.

METHODS

Ten obese nondiabetic male Zucker rats (OB) (fa/fa-) and 10 lean littermates (L) (Fa/+) were sacrified at 25 weeks of age according to Italian Ministry of Health Authorization (N°325/2015PR of 2015/04/05), heart and serum were collected. Serum IL33 and sST2 levels were assessed through ELISA. Molecular and western blot analysis of IL33 and ST2 were performed. Fibrosis deposition was evaluated on cardiac biopsies trough Sirious Red and western blot analysis of TGFbeta and collagen 3 protein levels

RESULTS

We showed that obesity determined a dysregulation of IL33/ST2 pathway characterized by a reduction of serum ($p=0.0627$) and cardiac ($p=0.0688$) IL33 and an increase of serum ST2 ($p<0.05$) in fat animals compared to lean controls. We showed increased TGFb ($p<0.05$) and collagen 3 ($p=0.0868$) protein levels in cardiac biopsies of fat animals, together with increased collagen fibers deposition ($p<0.05$ Sircol Red quantification). Finally we observed a dysregulation of EPAC1 expression in cardiac biopsies of fat animals.

CONCLUSIONS

We observed fibrotic remodeling of cardiac tissue and EPAC1 dysregulation in cardiac biopsies of fat animals, suggesting that obesity promotes cardiac remodeling through the loss of IL33/ST2L cardioprotective pathway

P128

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

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BACKGROUND-AIM

PDAC is one of the cancers with worst prognosis. This occurs because PDAC is often diagnosed at advanced stages, and because of an intrinsic resistance to the most common chemotherapy. Advanced stage PDAC is treated primarily with gemcitabine, known to be metabolically unstable. To compensate, it is administered in high doses, which generates serious side effects. To overcome these problems, we generated an orthotopic xenograft mouse model in which we tested the antineoplastic efficacy of a new engineered bifunctional antibody directed against the molecular target hERG1/β1 integrin complex (scDb) which has been proven to affinity target PDAC cancers, in combination with Gemcitabine (GEM).

METHODS

The orthotopic PDAC mouse model was developed by USGI of 1 X 106 PANC-1 cells on the pancreas of athymic nude mice. A 20 µL bolus of cells suspended in PBS was injected directly into the pancreas using a 50 µL Hamilton syringe with a 27 g needle previously placed in the mechanical syringe holder and lined up parallel to the US-transducer and perpendicular to the body. VevoLAZR-X system was used for USGI and for monitoring the tumor development. 4 groups of treatment were made:1) CTRL (saline); 2) GEM 0.5 mg/mouse; 3) scDb; 4) 320 mg/mouse;4) GEM 0.1 mg/mouse + scDb 320 mg/mouse. GEM was administered i.p. 3 times per week; scDb was administered i.v. every day.

RESULTS

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

CONCLUSIONS

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

P129

CUES OF ANTI-INFLAMMATORY ACTIVITY INDUCED BY CANNABIDIOL (CBD) ON THE MONOCYTE/MACROPHAGE THP-1 CELL LINE.

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BACKGROUND-AIM

Introduction: Cannabidiol (CBD) is the main non-psycho-active component of the Cannabis sativa. Like other psycho-active cannabinoids (i.e. THC), CBD is an immune-modulating agent that affects T cells, B cells, macrophages and microglia cells. Several studies have shown the cannabinoids' ability to suppress inflammatory response, reducing pro-inflammatory cytokine expression and increasing levels of anti-inflammatory cytokine. In order to investigate mechanisms related to the immunomodulation triggered by cannabinoids, this study analyses the anti-inflammatory ability of CBD to affect immunocompetent cells using the THP-1, an established leukemic monocytic lineage, able to differentiate into macrophages upon PMA treatment.

METHODS

Methods: THP-1 cells pre-activated with PMA (1 µg/mL) for 3 days according to the procedures, were pre-incubated with CBD 0.3 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 analysed using the ELISA assay.

RESULTS

Results: As expected LPS treated macrophages increased their pro-inflammatory IL-6 expression. Pre-treating cells with CBD reduce the IL-6 expression and increase IL-10 expression. These variations in inflammatory interleukins levels are more significantly after 48 hours and for cells treated with the higher concentration (1 uM) of CBD.

CONCLUSIONS

Conclusions: The cells treated with CBD showed a reduced expression of pro-inflammatory IL-6 and an increasing of anti-inflammatory IL-10. These findings confirmed CBD anti-inflammatory ability, suggesting that it may have therapeutic potential for inflammatory diseases.

P130

NOGO-B AND VERSICAN PROTEOGLYCAN AS NEW LINKS BETWEEN BREAST CANCER AND METABOLIC DYSFUNCTIONS

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BACKGROUND-AIM

Tumor microenvironment plays an important role in supporting tumorigenesis. In breast cancer, adipose tissue represents a large part of the microenvironment where metabolic alterations associated to obesity and/or diabetes (insulin resistance, hyperglycemia, low-grade inflammation) may affect cellular functions and modify the interactions between cancer cells and the stroma. Neurite Outgrowth Inhibitor-B (Nogo-B) and proteoglycan Versican (VCAN) have been associated to different types of human cancers, however, their role in human breast cancer is still unclear. Here, we have assessed their role as novel mammary adipose tissue factors involved in adipose-tumor bidirectional signals.

METHODS

Nogo-B and VCAN expression have been evaluated in human mammary adipose tissues (MAT) obtained from healthy women, in MAT-derived mesenchymal stem cells (MSC), and in breast cancer tissues. Total RNA has been obtained and analysed by qPCR. Nogo-B and VCAN content was investigated in MAT by immunohistochemistry (IHC) in peri-tumoral area of human breast cancer samples.

RESULTS

Nogo-B was expressed in MAT specimens and its mRNA levels positively correlated with fasting glucose and triglycerides levels. Nogo-B was also detected in mammary peritumoral adipose tissue by IHC. Interestingly, Nogo-B receptor mRNA levels positively correlated with both glycemia and body mass index (BMI) in breast cancer tissues. VCAN mRNA levels did not display significant correlation in MAT, but positively correlated with glycemia in MAT-derived MSC. Moreover, VCAN was detected in peritumoral MAT biopsies by IHC at higher extent in the proximity of the tumor, compared to more distant sites.

CONCLUSIONS

Nogo-B and VCAN were expressed in MAT and correlated to metabolic conditions. Thus, Nogo-B and VCAN could represent a crucial link between breast cancer and metabolic disorders. Characterization of VCAN and Nogo-B in MAT and Nogo-B receptor in breast cancer may lead to identify novel diagnostic biomarkers of breast cancer aggressiveness in adipose tissue.

P131

BAFF, BAFF PROMOTER AND BAFF RECEPTOR ALLELIC VARIANTS IN HCV RELATED CRYOGLOBULINEMIC VASCULITIS AND NON-HODGKIN'S LYMPHOMA

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BACKGROUND-AIM

The B-cell activating factor (BAFF) plays an essential role in the pathogenesis of lymphoproliferative disorders (LPDs), including those HCV-related. The increasing in BAFF levels associated with LPDs seems to be attributable to single nucleotide polymorphisms (SNPs) in the BAFF gene, BAFF promoter (BAFF-P) and BAFF receptor (BAFF-R).
Previous data suggested that SNP rs9514828 (-871C/T) on the BAFF-P as well as rs12428930 on the BAFF gene are associated with LPDs.
In addition, the His159Tyr mutation on BAFF-R seems to contribute to NHL onset through the NF-kB pathway activation, which induces apoptosis inhibition and magnifies all the BAFF effects.
The aim of this study was to evaluate how BAFF/BAFF-R SNPs can predispose to HCV-related cryoglobulinemic vasculitis (CV) and B-cell Non-Hodgkin's Lymphoma (NHL) and to establish a translational value for risk assessment and personalized medicine.

METHODS

416 HCV-chronically infected patients were tested: 136 HCV without LPDs (HCV), 166 HCV with CV (HCV-CV) and 114 HCV with NHL (HCV-NHL). The rs9514828 on BAFF-P, the rs61756766 on BAFF-R and the rs12428930 on the BAFF gene were studied by Real-Time PCR.

RESULTS

Concerning rs9514828, the frequency of C/T genotype was significantly higher in HCV-CV than in HCV. The difference in the distribution of the T/T mutant genotype in HCV-CV vs HCV was significant as well as the distribution of C/T and T/T genotype in HCV-NHL vs HCV. T minor allele was more frequent in HCV-NHL and HCV-CV than in HCV. The distribution of C/T+T/T (for the dominant model of penetrance C/T+T/T vs C/C) was significantly higher in HCV-CV and HCV-NHL than in HCV. Genotyping of rs61756766 SNP on BAFF-R, revealed C/T heterozygosis at a frequency of 11% in HCV-NHL vs 3% in HCV. The T minor allele frequency was higher in HCV-NHL than in HCV.
No differences emerged by genotyping rs12428930 SNP on BAFF coding gene.

CONCLUSIONS

Our data reinforce the hypothesis that BAFF/BAFF-R genetics has a role in the pathogenesis of HCV-related LPDs. BAFF/BAFF-R variants could identify a risk haplotype for HCV-related LPDs and a genetic profile assessment could potentially contribute to tailoring anti-BAFF (Belimumab) therapy by identifying patients with BAFF alterations in which it could be more beneficial.

P132

P2X7 RECEPTOR PROMOTES METASTATIC SPREADING AND INDUCES EXTRACELLULAR VESICLES RELEASE FROM MELANOMA CELLS

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BACKGROUND-AIM

Metastatic spread is the main cause of death in melanoma patients. It is noted that vesicles released from cancer cells are involved in cellular communication, tumor growth promotion and metastasis formation. It is well known that P2X7 is involved in cancer proliferation, angiogenesis and metastasis and its stimulation induces the release of vesicles from neural and immune cells. Therefore, we investigated the role of P2X7 in metastasis dissemination and vesicles release in a melanoma model.

METHODS

The expression of P2X7 was evaluated in human melanoma samples by immunohistochemistry and RT-PCR. We also studied the effect of P2X7 antagonism on anchorage-independent growth, migration, in vivo dissemination and lung metastasis formation in melanoma models obtained with human Ma-Mel-19 and Sk-Mel-28, and murine B16, melanoma cells. The release of vesicles from P2X7 stimulated cells were observed by confocal and electron microscopy and the different vesicle fractions were characterized by nanoparticle tracking analysis and western blot. The miRNA content was investigated through next-generation sequencing and RT-PCR.

RESULTS

We observed an association between P2X7 overexpression and melanoma progression. Moreover, P2X7 antagonists reduced melanoma dissemination both in vitro and in vivo experiments. We showed a P2X7-dependent secretion of microvesicles and exosomes from melanoma cells. Furthermore, the stimulation of P2X7 modified the miRNA content of both vesicle fractions causing the up-and down-modulation of around 200 miRNAs. miR-376c-3p, miR-495-3p and miR-6730-3p were up-modulated in both microvesicles and exosomes, and their expression was reduced by P2X7 antagonism. When transfected on melanoma cells, miR-495-3p and miR-6730-3p favor cell proliferation, while miR-376c-3p supports cells migration.

CONCLUSIONS

This data suggest a possible role of P2X7 in melanoma dissemination probably due to vesicles release and makes P2X7 a potential pharmacological target for advanced melanoma treatment.

P133

ABSENCE OF HOST-P2X7R FAVOURS IMMUNOSUPPRESSION, NEOVASCULARIZATION AND A2AR EXPRESSION IN THE TUMOR MICROENVIRONMENT

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BACKGROUND-AIM

In tumor microenvironment extracellular ATP and P2X7 receptor (P2X7R) play a central role in cancer growth and progression. ATP and adenosine recently emerged as main constituents of the tumor niche where they exert opposite and complementary roles. ATP promotes tumor growth but also immune eradicating responses, while adenosine is a potent immune suppressor. Blocking the CD73 or the adenosine A2A receptor (A2AR) activity relieves immunosuppression resulting in restraint of tumor growth. Similarly, blockade of the P2X7R causes an important reduction in experimental cancer rise. However, although both mechanisms have been investigated in preclinical models, studies analyzing the interplay between the P2X7R and the adenosinergic system in oncology are still missing.

METHODS

Using an animal model of melanoma that overexpress P2X7R and is sensitive to purinergic modulators, we performed in vivo and ex vivo experiments to study the role of P2X7R and adenosinergic system during tumor progression. We analyzed a panel of the main pro-inflammatory cytokines in tumor-bearing P2X7R null mice compared to wild type. We also investigated the expression of A2AR in the same mice, treated or not with an A2AR antagonist. The effect of P2X7R absence on A2AR expression and blood vessels formation was also analyzed by immunohistochemistry.

RESULTS

Cytokines' analysis shows that there is a reduction of systemic levels of proinflammatory cytokines and an increase of TGFβ in tumor-bearing P2X7R null mice compared to wild type. We then investigated the expression of A2AR in tumors derived from wild type and P2X7R null mice and in the presence or absence of the A2AR antagonist SCH-58261, showing a significant increase of the receptor in P2X7R deleted mice, confirming that P2X7R deficiency favors immune suppression. Immunohistochemical analysis of tumors derived from P2X7R null mice reveals also an augmented blood vessels formation, accompanied by an increase of systemic levels of VEGF.

CONCLUSIONS

During oncogenesis, A2AR has an immunosuppressive and neovascularizing effect in the absence of P2X7R. Inhibition of both receptors may represent a possible pharmacological target to promote antitumor immune response and reduce neovascularization in the tumor microenvironment.

P134

ROLE OF MIR-331-5P IN THE PATHOGENESIS OF THYROID CANCER

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BACKGROUND-AIM

Although the majority of thyroid cancer patients respond well to therapy, no effective therapeutic strategies are available for the anaplastic thyroid cancer (ATC) subtype that results to be one of deadliest malignancy. In this field, microRNAs (miRNAs), regulating gene expression, could play an important role in the molecular pathogenesis of thyroid cancer and could be used as novel therapeutic targets and prognostic factors. Recent studies showed that the members of miR-331 family (including miR-331, miR-331-3p and miR-331-5p) are altered in several human cancer but their role in thyroid cancer remain unclear. Here, we aim to characterize the role of miR-331-5p in the progression of thyroid cancer

METHODS

The expression levels of miR-331-5p and of its predicted mRNA target in thyroid cancer tissues were examined by quantitative-RT-PCR (q-RT-PCR). Stable transfections were performed to study the biological effects of the ectopic expression of miR-331-5p in anaplastic and papillary thyroid cancer cell lines. Cancer cell proliferation, migration and invasion were monitored by cell count, wound healing and Matrigel matrix assay, respectively. Luciferase assay and western blot analysis were performed to investigate the possible binding of miR-331-5p to its target predicted.

RESULTS

miR-331-5p is significantly downregulated in thyroid cancer tissues compared to normal thyroids. Ectopic expression of miR-331-5p in thyroid cancer cells reduced their motility phenotype and lead to the downregulation of BID protein. Importantly, the luciferase assay confirmed the direct targeting.

CONCLUSIONS

Overall, our findings suggest that deregulation of miR-331-5p could promote the aggressiveness of thyroid cancer cell lines, representing a novel potential candidate biomarker and target for this malignancy.

P135

THE RNA-BINDING UBIQUITIN LIGASE MEX3A AFFECTS GLIOBLASTOMA TUMORIGENESIS BY INDUCING UBIQUITYLATION AND DEGRADATION OF RIG-I

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BACKGROUND-AIM

Glioblastoma multiforme (GB) is the most malignant primary brain tumor in humans, with an overall survival of approximatively 15 months. The molecular heterogeneity of GB, as well as its rapid progression, invasiveness, and the occurrence of drug-resistant cancer stem cells, limit the efficacy of the current treatments. In order to develop an innovative therapeutic strategy, it is mandatory to identify and characterize new molecular players responsible for the GB malignant phenotype.

METHODS

In this study, the RNA-binding ubiquitin ligase MEX3A was selected from a gene expression analysis performed on publicly available datasets, to assess its biological and still-unknown role in GB tumorigenesis. In order to identify the mechanism of action of MEX3A and its potential interactors in GB, several biochemical and biological assays were been performed

RESULTS

We find that MEX3A is strongly up-regulated in GB specimens, and this is associated with very low protein levels of the Retinoic acid-inducible gene I (RIG-I), a tumor suppressor involved in the activation of innate immune response and in the induction of cell growth arrest via apoptosis. We demonstrated that MEX3A binds RIG-I and promotes its ubiquitylation and proteasome-dependent degradation. Furthermore, the genetic depletion of MEX3A leads to an increase of RIG-I protein levels, resulting in a significantly decrease of GB cell growth both in vitro and in vivo.

CONCLUSIONS

Our findings unveil a novel molecular mechanism involved in GB tumorigenesis and suggest MEX3A and RIG-I as promising therapeutic targets in GB.

P136

COMPARISON OF TWO METHODS TO QUANTIFY INFLIXIMAB AND ADALIMUMAB SERUM CONCENTRATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES.

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BACKGROUND-AIM

Inflammatory bowel diseases (IBD) are a group of complex disorders characterized by immune mediated chronic intestinal inflammation. Currently, anti-tumor necrosis factor-α biological drugs are first line therapies for patients with severe IBD.
Although such biological therapies present in general a good efficacy, primary or secondary loss of response occurs in 20-50% of patients.
Loss of response may be due to pharmacodynamic and/or pharmacokinetic issues (subtherapeutic drug concentrations or development of anti-drug antibodies-ADA).
Thus, therapeutic drug monitoring (TDM) may improve the management of patients with IBD. Fast and reliable results of TDM may allow rapid intervention to avoid toxicity and improve efficacy.
The aim of this study was to assess the analytical performance of a new fully automated chemiluminescent assay to measure biological drugs and ADA serum concentration as compared to the ELISA methods currently used in our laboratory.

METHODS

Serum levels of biological drugs (Infliximab and Adalimumab) and their respective ADA were measured in 20 IBD patients treated with infliximab (IFX) and 20 IBD patients treated with adalimumab (ADL) using the Promonitor® ELISA kits (Grifols) and the i-TRACKER chemiluminescent (CLIA) kits (Theradiag).

RESULTS

A very good correlation was observed both for IFX (Spearman r=0.9695, 95%CI = 0,92-0,99, p<0.0001) and ADL serum levels (Spearman r=0.9476, 95%CI = 0,86-0,98, p<0.0001). Among the patients with undetectable IFX serum levels, 6/8 resulted ADA positive by ELISA versus 8/8 by CLIA. Moreover, in 3 cases CLIA disclosed very high ADA concentration compared to borderline results by ELISA. As regards to ADL, all the 9 patients with undetectable serum levels presented similar ADA concentration as tested with ELISA or CLIA.

CONCLUSIONS

In conclusion, in our study, the i-TRACKER CLIA infliximab and adalimumab assays showed a very good correlation with the ELISA methods for quantitative levels of drugs, furthermore a good agreement of qualitative results was observed also for ADA, with a higher sensitivity for ADA against IFX by CLIA compared to ELISA. The use of a fully automated assay for TDM would significantly improve the management of IBD patients also in terms of short turn-around time.

P137

MICROVESICLES DERIVED FROM THYROID CANCER CELLS PROMOTE THE EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) AND THE TRANSFER OF MALIGNANT PHENOTYPES.

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BACKGROUND-AIM

Thyroid cancer is the most common endocrine cancer with predominant prevalence of papillary thyroid cancer (PTC) histotype. A small percentage of papillary tumors is associated with metastases and aggressive behavior. This is due to de-differentiation obtained through the EMT through which epithelial thyroid cells acquire a fibroblast-like morphology, reduce cellular adhesion, increase motility and expression of mesenchymal proteins. The tumor microenvironment plays an important role on promoting a more or less aggressive phenotype through hypoxia and the secretion of HMGB1 and other factors. In particular, hypoxia has been shown to change drastically the phenotype of tumor cells and it has been associated with increasing metastatic and migratory behavior. In addition, cells also release extracellular membrane vesicles containing key molecules that allow to transfer information within the tumor and microenvironment.

METHODS

CAL 62 microvesicles were isolated by ultracentrifugation and characterized through CD81 expression. Microvesicles RNA cargo expression (microRNA and mRNA) was determined by qRT-PCR. The change in phenotype was evaluated through the detection of several proteins and microRNAs that are known to be induced during EMT. Moreover, HMGB1 expression was studied by ELISA in both supernatants and microvesicles extracted from BC PAP and CAL 62 cultures.

RESULTS

In this work we demonstrate that microvesicles produced by CAL 62 contain HMGB1, miR221, 222 as well as miR9 and miR200a/c that are involved in hypoxic response and EMT. BC PAP treated with CAL 62 microvesicles showed an increase in HIF1a and EMT markers such as YAP and Vimentin. In addition, HMGB1 mRNA and protein were found in microvesicles from CAL 62 cells.

CONCLUSIONS

Since addition of microvesicles from CAL 62 to BC PAP cells induces overexpression of HIF1a and EMT markers, we can propose that malignant phenotypes can be transferred from cell to cell within the same tumor and /or to cells in distant sites through the production of Microvesicles.

P138

NUTRIENT DEPRIVATION PROMOTES THE GENERATION OF AN ATP-RICH TUMOR MICROENVIRONMENT BY ENHANCING RELEASE OF ATP-LOADED MICROPARTICLES: IMPLICATIONS FOR THERAPY

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BACKGROUND-AIM

Caloric restriction has been shown to reduce tumor incidence and caloric restriction mimetics (CRM) enhance the anticancer activity of known chemotherapics. Recently, it has been proposed that this might be due to CRM ability to increase the ATP concentration in the tumor microenvironment (TME), modifying the TME composition. The TME is rich in extracellular ATP (eATP), that acts at plasma membrane P2 receptors, among which the P2X7 receptor (P2X7R) subtype is pivotal for its role in inflammation and cancer. P2X7R is a promoter of cell survival or of cell death depending on the activation level. Furthermore, it is a main sensor of the eATP concentration in the TME and a conduit for ATP release. Despite increasing awareness of the key importance of eATP in the TME, the relationship between cancer cell metabolism and eATP, as well as the cellular mechanisms involved in eATP accumulation in the TME, are as yet unknown.

METHODS

We induced caloric restriction in vivo, by CRM administration to C57bl/6 WT mice, and in vitro, by exposing B16F10 mouse melanoma cells to serum starvation and CRM treatment. Intracellular and extracellular ATP concentrations were measured using soluble luciferase and plasma membrane-expressed luciferase (pmeLUC). Mitochondria were analysed with the SeaHorse apparatus and confocal microscopy. Microparticles were investigated using a NanoSight instrument, electron microscopy and western blot analysis.

RESULTS

In vivo CRM administration or in vitro serum starvation reduced melanoma tumor size and inhibited melanoma cell growth, and simultaneously caused a large increase in eATP levels. Chronic incubation in the absence of serum severely impaired mitochondrial energy metabolism and dramatically reduced iATP levels. At the same time, serum deprivation stimulated a P2X7 receptor-dependent release of ATP-loaded, mitochondria-containing, microparticles, and of naked mitochondria vesicles.

CONCLUSIONS

In conclusion our findings suggest a mechanism by which nutrient deprivation causes impairment of cancer cell metabolism and generates an eATP rich TME. These observations provide further rationale to anticancer therapies based on caloric restriction or on the administration of caloric restriction mimetics.

P139

LIFESTYLE FACTORS AFFECT MULTIPLE CYTOKINES IN HEALTHY INDIVIDUALS

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BACKGROUND-AIM

Low-grade chronic inflammation (LGCI) is a common characteristic of many noncommunicable diseases, such as obesity, type 2 diabetes, cardiovascular disease, chronic respiratory disease, and cancer. Cytokines play a crucial role in LGCI. This study aimed to assess how LGCI risk factors (age, body mass index -BMI, smoke, physical activity and diet) may impact on specific cytokine levels in a healthy population.

METHODS

150 healthy volunteers were recruited and subjected to questionnaires about the last 7-days lifestyle, including smoke habit, physical activity, and food frequency. A panel of circulating cytokines, chemokines, and growth factors was analyzed by multiplex ELISA assay.

RESULTS

BMI showed the heaviest impact on the correlation between LGCI-related risk factors and cytokines and was significantly associated with CRP levels. Aging was characterized by an increase of IL-4, IL-7, IL-13, eotaxin, MCP-1, and MIP-1α. Smoking was related to higher levels of PDGF, IL-1ra, and CCL5/RANTES, while physical activity to MIP-1α. Within the different eating habits, subjects with both the highest pasta/cereals and meat consumption displayed the most inflammatory pattern, characterized by the increase of multiple cytokines, including IL-6. IL-6 levels were also increased with the frequent intake of pasta/cereals in combination with sweets.

CONCLUSIONS

In conclusion, age, BMI, smoke, physical activity, and dietary habits are associated with specific cytokines, which represent potential markers for LGCI.

P140

OLIVE PHENOLS PRESERVE LAMIN B1 EXPRESSION REDUCING CGAS/STING/NFκB-MEDIATED SASP IN IONIZING RADIATION-INDUCED-SENESCENCE.

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BACKGROUND-AIM

Cellular senescence is a state of terminal growth arrest in which cells are irresponsive to growth factor stimulation. Senescence occurs upon critical telomere shortening (replicative senescence) or following DNA damage, oncogenic activation, hypoxia and oxidative stress, overall referred to as Stress induced Premature Senescence (SIPS).
In response to DNA damage, cells release cytoplasmic chromatin fragments (CCFs). Senescent cells do not divide, though remaining metabolically active and generate an altered secretome, termed the Senescence-Associated Secretory Phenotype (SASP). SASP contributes to generate a pro-inflammatory and pro-tumoral extracellular milieu. Recent studies have shown the protective role of polyphenols, owing to their anti-inflammatory and anti-tumor activities.
Here, we studied the effects of two polyphenols, oleuropein aglycone (OLE) and hydroxytyrosol (HT) on DNA damage, CCFs appearance and SASP in a model of irradiation-induced senescence.

METHODS

Neonatal human dermal fibroblasts (NHDFs) were γ-irradiated.
After irradiation, the NHDF cells were subjected to treatment with oleuropein (5μM) and hydroxytyrosol (1μM), for 2 weeks (5 treatments).
Cell growth and Senescence-Associated (SA)-ß-GAL staining were used as senescence markers. DNA damage and nuclear stability were evaluated by Comet Assay. Lamin B-1 expression, CCFs release, cyclic GMP-AMP Synthase (c-GAS) and NFKB activation were evaluated by immunofluorescence protocol.
Fibroblast-conditioned media (CMs) were collected and analyzed for IL-6, IL-8, MCP-1 and RANTES by ELISA assay.

RESULTS

Our results showed that 8 Gy irradiation was effective in inducing premature senescence and that treatment with OLE and HT exerted a protective effect, preserving Lamin B1 expression and reducing CCFs.
We also demonstrated a reduction of c-GAS/Stimulator of Interferon Genes (STING) activation, NFkB nuclear localization and SASP factor release. After treatment with OLE and HT, we observed a reduction of IL-6 and IL-8, MCP-1 and RANTES release in extracellular medium was observed in γ-irradiated-NHDFs.

CONCLUSIONS

The ability of polyphenols to mitigate DNA damage, senescence status and the related SASP in normal cells can be exploited to improve the efficacy and safety of cancer radiotherapy.

P141

COOPERATIVE EFFECT OF RADIOTHERAPY AND GOLD NANOPARTICLE-INDUCED HYPERTHERMIA IN CANCER TREATMENT

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BACKGROUND-AIM

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

METHODS

The long-term cytostatic/cytoxic effects of combined radiotherapy and nano-mediated hyperthermia on breast cancer and melanoma cell lines were evaluated using clonogenic assays while the short-term effects were determined evaluating DNA damage by western blot analysis of cleaved PARP and γH2AX and cell cycle arrest.

RESULTS

Our data show the cooperative effects of the lowest irradiation dose (2Gy) with nano-based hyperthermia in long term assay with significant reduction of colony number compared to the single treatment alone. Moreover, we observed increased levels of γH2AX and of inactivated PARP after the combo treatment. Based on preliminary studies, Endothelial colony Forming cells, (ECFCs), a subtype of Endothelial Progenitor Cells, were chosen to carry gold nanoparticles (AuNPs) to tumor cells and were used in co-culture experiments with unloaded melanoma cells. Our findings show that the excellent thermotrasductive properties of the cargo cells enhance the cytostatic effects of radiotherapy in the short term and long-term assays.

CONCLUSIONS

AuNPs are confirmed to be as excellent radiosensitizers and thus allows to shorten the duration of the treatment and to reduce the radiation doses. Nano-photothermal therapy increases and spatially delineates hyperthermic treatment. It is thus possible to delimit the radio-thermo-induced damage at the level of the tumor tissues and obtain greater efficacy from the therapeutic point of view. In the perspective of a clinical phase, the use of autologous ECFCs, promises to be an effective "personalized therapy" of cancer, with an almost zero risk of rejection.

P142

ASSESSMENT OF NEUTRALIZING, STRAIN-SPECIFIC HUMORAL IMMUNE RESPONSE TO SARS-COV-2 VACCINATION IN PATIENTS AFFECTED BY SYSTEMIC LUPUS ERYTHEMATOSUS TREATED WITH B-CELLS-TARGETED THERAPIES.

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BACKGROUND-AIM

Vaccination therapy against SARS-CoV-2 in rheumatologic patients receiving immunomodulant biologic drugs is challenging, since they may blunt the antibody response.
Aim of this study was to assess the development of a neutralizing, strain-specific response to SARS-CoV-2 vaccination in patients affected by Systemic Lupus Erythematosus treated with Belimumab (BLM, mAb anti-B-Lymphocytes stimulator BLyS).

METHODS

Patients receiving BLM (n=6) treatment were administered mRNA vaccination. Serum was collected ≈20 days after completion of the immunization scheme. A pseudovirus neutralization assay was conducted employing the target HEK293 T cell line overexpressing ACE2 and 2 different strains of GFP-expressing, replication deficient retroviruses, pseudotyped with the Spike protein of either the Wuhan-Hu1 strain or the B.1.1.7 strain. Incubation of the pseudovirus with serial dilutions of patients' sera before target cell seeding was carried out. Pre-pandemic sera (n=3), sera from vaccinated (n=3) healthy individuals and a neutralizing monoclonal antibody against the Spike protein were employed as negative and positive controls.

RESULTS

Patients receiving BLM were stratified according to total antibodies (IgG+IgM+IgA) against the receptor-binding domain (RBD) of the Spike glycoprotein into high responders (>800 AU/mL, n=3) and low responders (≤45 AU/mL, n=3) and tested with pseudovirus neutralization assay. Two thirds of low responders neutralized the Wuhan-Hu1 strain at medium titer (ID50: 10-3) but were ineffective in inhibiting the B.1.1.7 entry into target cells. Concerning high responders, while two patients were able to inhibit both the strains at medium-high titer (ID50: 10-4 for Wuhan-Hu1 and for10-3 B.1.1.7), one patient mainly neutralized the WT strain.

CONCLUSIONS

Although the majority of BLM treated patients were able to develop neutralizing antibodies against the Wuhan-Hu1 strain of SARS-CoV2, their ability to develop neutralizing antibodies against other, more prevalent, strains could be partially impaired.

P143

TARGETING ENDOTHELIAL CELL AND CANCER AMOEBOID MOVEMENT TO OVERCOME RESISTANCE TO ANTI-VEGF AND ANTI-PROTEASE THERAPIES

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BACKGROUND-AIM

Synthetic metalloprotease inhibitors (MPIs) were developed and utilized in human clinical trials for cancer therapy but the results were disappointing. The invasion strategy of single cells develops according to at least two distinct modes of migration: the elongated-mesenchymal and rounded-amoeboid. Unlike mesenchymal migration, the amoeboid motility doesn't require proteases and is an opportunistic movement of cancer cells that allows cells to glide through, rather than degrade, ECM barriers, using movements based on adaptations of the cell body. We hypothesized that the failure of cancer treatment using MPIs could be ascribed to the ability of cancer and also endothelial cells (ECs) to skip the attack of the MPI therapy by allowing cancer invasion and blood vessel formation using the "amoeboid" strategy.

METHODS

Experiments we conducted using a mixture of physiologic inhibitors of the main protease families, showed no differences in vessel formation for ECs under mesenchymal or amoeboid conditions. Thus, since ECs can move and differentiate in vascular organization in vitro also in absence of proteases, we hypothesized the existence of an "amoeboid angiogenesis". We have observed that even EC treatment with Marimastat, a broad-spectrum matrix metalloproteinase inhibitor, induced an increase of invasive cell number, cell proliferation and new vessel formation in vitro and in vivo. In cancer therapy even VEGFA targeting partially disregarded the expectations because of the resistance onset followed by the progression of the disease. Our experiments showed an "indifference" of ECs to VEGF stimulation using the mixture of physiologic protease inhibitors.

RESULTS

The results obtained could justify the failure of MPIs as cancer therapy that, at the initial stages of tumor development, could be ascribed also to the onset of the angiogenic transition, during which the tumor microenvironment is able to skip the attack of the MPI by allowing blood vessel formation using the "amoeboid" strategy. Moreover, the data shown can explain tumor resistance to VEGF-targeted therapies as result of the overcome of amoeboid cancer and endothelial cell behavior.

CONCLUSIONS

Targeting both VEGF and amoeboid movement in both endothelial and cancer cells, should result in more effective tumor growth inhibition.

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IN-VITRO ACTIVITY OF A SELECTIVE TKI INHIBITOR IN MEDULLARY THYROID CANCER

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BACKGROUND-AIM

Medullary thyroid cancer (MTC) is a rare malignant thyroid tumor arising from the neuroendocrine C cells with frequent mutations in the rearranged during transfection (RET) or RAS genes that result in aberrant activation of oncogenic signaling, such as PI3K/AKT pathway. Indeed, after surgery, patients with progressive disease are treated with the multi-targeted tyrosine kinase inhibitor, Cabozantinib. However, its long-term survival benefit is yet uncertain and adverse events are very common. Recently, the FDA approved a new selective RET inhibitor, Pralsetinib (Blu-667), for the treatment of both RET wild-type and mutant MTC. The aim of the study was the analysis of microRNA expression levels and other molecular aspects of MTC cells in hypoxic condition, used to increase cell aggressiveness, and after Blu-667 and Cabozantinib treatment.

METHODS

TT cells (MTC C634W RET mutated cells) were evaluated for microRNA expression levels before and after hypoxia using nCounter Sprint Profiler. In addition, TT cells were treated for 72h with 50 nM Pralsetinib (Blu-667) and/or 2.5 uM Cabozantinib in the presence or absence of hypoxia. Cell proliferation, apoptosis, RET and PI3K/AKT pathway activation were assessed.

RESULTS

We found 17 microRNAs induced by hypoxia in TT cells. Interestingly, Blu-667 was more effective than Cabozantinib in the inhibition of cell proliferation and induction of apoptosis and reverted TT cells resistance to Cabozantinib after hypoxia induction. Moreover, after Blu-667 treatment, we observed an inhibition of the phosphorylation of both RET and AKT, the latter being the readout of PI3K/AKT signaling.

CONCLUSIONS

In conclusion, TT cells under hypoxia are characterized by a microRNA signature that could have a role in the aggressiveness of tumors. Furthermore, we showed the in-vitro efficacy of Blu-667 in controlling both cell proliferation and cell death.

P145

MESOPOROUS SILICA NANOPARTICLES AS A VEHICLE FOR BORTEZOMIB TARGETED DELIVERY IN MULTIPLE MYELOMA

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BACKGROUND-AIM

One of the main challenges in cancer treatment is to develop a therapeutic strategy able to selectively target tumor cells preserving normal tissues from unwanted side effects. Localized drug delivery should cope this aim. A patented mesoporous silica-based nanodevice (EP 3 288 955 B1), bearing the antineoplastic drug bortezomib (BTZ), whose release is triggered by the acidic tumor environment, and grafted with the targeting function folic acid (FOL) on the external surface, was developed (FOL-MSN-BTZ) and tested in vitro and in vivo toward multiple myeloma (MM) cells and in xenograft models, respectively.

METHODS

FOL-MSN-BTZ efficacy studies were conducted through growth experiments, TEM, TUNEL assay and Western Blotting (WB). In vivo studies were performed on mice models bearing RPMI 8226 (RPMI) MM cells derived tumors.

RESULTS

FOL-MSN-BTZ was able to kill folate receptor overexpressing (FR+) cancer cells, but not FR- normal cells, while free BTZ was toxic for all cell lines tested, regardless of FR expression. MSNs uptake occurred exclusively in FR+ RPMI cells, through FR-mediated endocytosis, while no uptake was observed in FR- cells. Both FOL-MSN-BTZ and free BTZ led to comparable apoptotic rates in RPMI cells, but only BTZ caused also death in FR- normal cells. In mice bearing RPMI-derived tumors, the FOL-MSN-BTZ nanosystem resulted significantly more efficacious than the free drug, as soon as after the first administration.

CONCLUSIONS

These data show the outstanding specificity of FOL-MSN-BTZ against FR+ tumor cells, and together with the highly promising in vivo studies, allow a future exploitation of our MSNs technology for drug targeting applications, particularly in cancer therapy.

P146

A NOTCH INHIBITOR PLUS RESVERATROL DOWNREGULATES THE CD1/CDK4 SIGNALING SHAPING THE PHENOTYPE OF GLIOBLASTOMA CANCER STEM-LIKE CELLS

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BACKGROUND-AIM

Glioblastoma (GBM) is the most common, aggressive and lethal form of brain tumor in adults. The main cause of the failure of current therapeutic strategies is the presence, within the tumor mass, of a subset of cells with stem characteristics, called glioma stem cells (GSCs) which support carcinogenesis, establish resistance to the conventional therapy and determine relapse. Therefore, GSCs should be the main target for the development of new therapeutical strategies. Notch receptors are overexpressed in GSCs and the use of gamma secretase inhibitors (GSI) in the clinic is limited due to the severe side effects. Alternative therapy attempting to inhibit Notch signaling using phitochemicals combined with low doses of GSI would represent a novel promising strategy in GBM patient. Here we tested the effects of combined use of Resveratrol (RSV), a phytoalexin present in grapes, and low dose of GSI in glioblastoma cell lines.

METHODS

qRT-PCR, immunofluorescence (IF), western blotting assay, neurosphere formation efficiency (NFE), self-renewal (SR) and FACS analysis.

RESULTS

U87MG and T98G cells growth as neurospheres display an higher expression of CD1 and Cdk4 than in monolayer together with the expected markers of stem cells such as CD133, CD44 and Nestin. Furthermore the combined treatment with RSV and GSI after 24 hours, reduced CD1, Cdk4 and stem cell markers in both glioblastoma neurospheres compared with untreated cells. In addiction the co-treatment with RSV and GSI significantly inhibited the ability of U87MG and T98G cell to generate neurospheres and completely reset self renewal. FACS analysis revealed a reduction of CD44+ and CD133+ subpopulation in neurospheres co-treated respect to control.

CONCLUSIONS

This study highlights how the combined treatment with RSV and GSI drastically inhibits the overexpression of cyclin D1 and depend partner Cdk4, all target molecules involved in tumor stemness and in such-way may reduce glioblastoma cell growth and progression. All this addresses how this combined treatment could be employed in the novel therapeutic strategies of GBM patients.

P147

FOXO3A INHIBITS THE METASTATIC POTENTIAL OF TAMOXIFEN RESISTANT BREAST CANCER BY INDUCING INTEGRIN ALPHA 5 EXPRESSION

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BACKGROUND-AIM

Resistance to endocrine therapy is still a major clinical challenge in the treatment of estrogen receptor positive (ER+) breast cancer (BC) patients, whose poor outcome demands additional studies. Forkhead box class O (FoxO)3a transcription factor, a bona fide oncosuppressor, has been involved in BC metastasis as well as in antiestrogen resistance.

METHODS

FoxO3a and α5 cooperation and involvement in the reversion of BC motility and aggressiveness were assessed in vitro in different ER+ BC cell lines and their tamoxifen-derived cells by Western blot (WB), qRT-PCR, siRNA, Wound Healing Scratch assay, Transmigration and Invasion assays, Colony forming assay, Gene Reporter assay, Chromatin Immunoprecipitation (ChIP) assay, and in silico by cBioPortal Database.

RESULTS

Here we demonstrate that the α5 subunit of the integrin α5/ß1 a well-known membrane heterodimer controlling cell surface adhesion and signaling, is a novel FoxO3a transcriptional target.
FoxO3a re-expression reduces motility and aggressiveness of different Tamoxifen resistant BC cell lines, through the induction of α5 mRNA and protein levels. On the other hand, not only FoxO3a silencing, but also that of α5, restored basal motility and aggressiveness, confirming their involvement in this process. FoxO3a transcriptionally induces α5 expression by binding to specific Forkhead responsive elements located on the α5 promoter. Finally, using a large-scale BC gene expression datasets from The Cancer Genome Atlas (TCGA) database, a strong positive correlation between FoxO3a and α5 exclusively in ER+ BC patients emerged.

CONCLUSIONS

Altogether, our data unveil an additional mechanism through which FoxO3a activation/induction, by increasing α5 expression, restores a less aggressive phenotype in BC refractory to endocrine therapy.

P148

MIR23A AND MIR29A AS PREDICTIVE BIOMARKERS OF EARLY-STAGE PERIPHERAL MAMMARY CARCINOGENESIS AND BONE MARROW REMODELING

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BACKGROUND-AIM

Cancerogenesis represents the outcome of a dynamical and reciprocal interaction between transforming cells and the surrounding microenvironment. The cross-talk between cancer cells and components of the tumour microenvironment plays a critical role in cancer development, survival, progression, metastasis and resistance to therapy.

METHODS

Transgenic NeuT+ mice represent a powerful model for studying all stages of breast cancer development and progression. Simultaneous morphological analysis of peripheral mammary lesions (dysplasia, in situ carcinoma, invasive carcinoma) and bone marrow samples allowed correlating the development of the neoplastic clone, the adaptation of the marrow haematopoietic response and the variations in circulating miRNAs.

RESULTS

Hematopoietic marrow of 12 weeks old NeuT+ mice, corresponding to in situ cancer stage, showed expansion of granulocytic myeloid cells and contraction of lymphoid and erythroid compartments, as compared with BALB/c controls. The same alterations, though less prominent, were also observed in the bone marrow of 6 weeks NeuT+ mice, in which the mammary glands display pre-cancerous lesions (i.e. moderate-to-severe epithelia dysplasia), proving that an incipient neoplasia influences the bone marrow by switching tumor-adapted hematopoiesis. To define circulating miRNA signatures/clusters specifically associated with the different stages of the disease, we analyzed the levels of miRNAs along time. Profiling of plasma miRNAs revealed differentially expressed miRNAs including miR-29 and miR-23 cluster elements that modulate extracellular matrix synthesis, the CXCL12/CXCR4 axis and B cell receptor programs. Furthermore, these miRNAs were closely correlated with the overall tumor burden of mice at 12 weeks, fueling the hypothesis of an influence of cancer on circulating miRNA levels.

CONCLUSIONS

This evidence represents a potential link between peripheral transformation and hematopoietic adaptation of the bone marrow through circulating miRNAs and suggests that circulating miRNAs associated with breast cancer can also be detected in pre-invasive conditions, representing an important point for translation the human setting.

P149

CARBONIC ANHYDRASE IX AS A POTENTIAL THERAPEUTIC TARGET IN CHEMORESISTANT GASTRIC CANCER

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BACKGROUND-AIM

Gastric cancer (GC) represents the fourth leading cause of cancer-related death worldwide. Due to the low rate of early diagnosis and the lack of specific signs, chemotherapy currently represents the only therapeutic option for advanced GC, but chemoresistance onset strongly affects patients' outcomes. Therefore, the identification of new suitable targets to restore the acquired drug sensitivity is of fundamental importance. The Carbonic Anhydrase IX (CAIX), a tumor-associated cell-surface glycoprotein involved in the metabolic adaptation of several histotypes of cancer cells, is also a major key mediator of drug resistance and tumor progression. This study aims to elucidate whether the CAIX might be associates with GC resistance and whether the CAIX inhibitor SLC-0111 may overcome this resistance.

METHODS

Through an ad-hoc in vitro model of experimental-induced chemo-resistant AGS cell line established in our laboratory, we observed the CAIX expression in 5-Fluorouracil-resistant (5-FUr) and Paclitaxel-resistant (TAXr) GC cells. We exploited the Seahorse platform to obtain a complete metabolic profile of the chemoresistant cells and consequently, we investigated the effect of the CAIX inhibitor SLC-0111 through caspase activation and viability assays.

RESULTS

5-FUr and TAXr cells are characterized by a high expression of CAIX and by a boosted glycolytic metabolism with subsequent extracellular acidification, which may account for their increased CAIX expression. We then demonstrated that the SLC-0111 treatment is able to halve the proliferation of AGS control cells and even significantly potentiate the efficacy of 5-FU or Paclitaxel treatments. More interestingly, the SLC-0111 treatment inhibited the proliferation of 5-FUr and TAXr cells, and when used in combination with 5-FU or Paclitaxel, we found that their efficacy was significantly strengthened. The proliferative block exerted by SLC-0111 in AGS cells, either in control and resistant cells is paired with the induction of the apoptotic program.

CONCLUSIONS

The CAIX may represent a promising target in chemoresistant GC and the CAIX inhibitor SLC-0111 might serve as an anti-cancer adjuvant agent to be used in a novel complementary therapeutic strategy for advanced GC.

P150

METFORMIN INHIBITS GLIOBLASTOMA CELL PROLIFERATION: IS SURVIVIN A PROMISING TARGET?

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BACKGROUND-AIM

Glioblastoma (GBM), also referred to as a grade IV astrocytoma, is the most frequent and aggressive infiltrative brain tumor in adults >50 years of age. The poor prognosis, with a median survival of 15-17 months after diagnosis, is mainly linked to the occurrence of chemo- and radio-therapy resistance. Therefore, new therapies or drug repurposing are an urgent need. In order to highlight additional mechanisms through which the anti-diabetic drug Metformin interferes with GBM growth, we focused our attention on its action on survivin, a member of the inhibitor of apoptosis (IAP) family and a key factor in GBM cell spreading and chemo-therapy resistance acquisition.

METHODS

Protein expression was evaluated by western blotting (WB) assay; cell viability was investigated by MTT assay; cell proliferation was analyzed by trypan blue exclusion, colony formation and anchorage-independent soft agar assays.

RESULTS

Metformin inhibited cell viability and proliferation of U87MG and T98G GBM cell lines. Moreover, following Metformin treatment, a lower anchorage-dependent and independent growth ability was observed by colony formation and soft-agar assays, respectively. These events were paralleled by 1) the hyper-activation of the AMPK signaling and the inhibition of the AKT and mTOR pathways which are well-known mechanisms of metformin anti-tumor activities, 2) modulation of FOXO3a nuclear translocation, and 3) changes in survivin expression. The effect of Metformin on survivin expression has never been described before. Here we show, a significant reduction of survivin protein levels in both metformin-treated U87MG and T98G cells, unveiling an additional mechanism involved in the anti-cancer activity of metformin in GBM.

CONCLUSIONS

The anti-diabetic agent Metformin is able to efficiently suppress growth and survival in different GBM cell lines. Investigation unreveling molecular mechanism involved in Metformin modulation of AMPK/FOXO3a/survivin pathway, will be further conducted. However, these preliminary results might pave the way for a future exploitation of Metformin as a repurposing drug in the adjuvant therapy of GBM.

P151

VALIDATION OF A SALIVA-BASED TEST FOR THE MOLECULAR DIAGNOSIS OF SARS-COV-2 INFECTION

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BACKGROUND-AIM

During the peak of the COVID-19 pandemic, clinicians and researchers have been searching for new alternative tests to improve screening and diagnosis of SARS-CoV-2 infection. Currently, the gold standard for virus molecular identification is the nasopharyngeal (NP) swab. Saliva samples, however, offer clear practical and logistical advantages in terms of procedure tolerance, number of medical personnel involved and testing sites, but due to lack of collection, transport and storage solutions, high-throughput saliva-based laboratory tests are difficult to scale up. With this study, we aimed to validate a molecular detection method for SARS-CoV-2 on saliva collected in a new storage and inactivating solution, comparing the results to NP swabs to determine the difference in sensitivity between the two tests.

METHODS

In this study, 156 patients and 1005 asymptomatic subjects were enrolled and tested simultaneously for the detection of the SARS-CoV-2 viral genome by RT-PCR, on both NP swab and saliva. Saliva samples were collected in a preservative saline solution. An internal method validation was performed to standardize the entire workflow for saliva samples processing.

RESULTS

The identification of SARS-CoV-2 on saliva samples showed a clinical sensitivity of 95.1% and specificity of 97.8% compared to NP swabs. The positive predictive value (PPV) was 81% while the negative predictive value (NPV) was 99.5%. Test concordance was 97.6% (Cohen's Kappa=0.86; 95% CI 0.81-0.91). The LoD was 5 viral copies for both samples

CONCLUSIONS

RT-PCR assays conducted on preserved saliva samples achieved similar performance to those on NP swabs and this may provide a very effective tool for population screening and diagnosis. Collection of saliva in a stabilizing solution makes the test more convenient and widely available; furthermore, the denaturation properties of the solution reduces the infective risks belonging to sample manipulation.

P152

THE KCTD1 PROTEIN NEGATIVELY REGULATES THE HEDGEHOG SIGNALING THROUGH INTERACTION WITH THE ONCOSUPPRESSOR KCASH2 AND ITS STABILIZATION.

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BACKGROUND-AIM

KCASH2 belongs to the KCASH family of proteins, involved in the negative regulation of Sonic Hedgehog (Hh) pathway. KCASH2 induces HDAC1 degradation, inhibiting transcriptional activity of Gli1, the main target and effector of the Hh pathway. Recently, has been discovery KCTD15 which, through its interaction with and stabilization of KCASH2, is involved in the Hh pathway modulation. KCTD15 is capable to enhance KCASH2 inhibitory effect in Medulloblastoma (MB) cells, reducing tumor cell growth. Coherently, KCTD15 expression is reduced in a percentage of human sporadic MB which present Hh hyperactivation.
KCTD15 shares several features and biological functions with its paralogue KCTD1. This homology might suggest that also KCTD1 is involved in the regulation of KCASH2 and modulation of Hh pathway.

METHODS

KCTD1 interaction with KCASH2 and KCTD15 was verified by co-immunoprecipitation assays. WB analysis have been used to evaluate KCTD1 roles on KCASH2 and KCTD15 protein stability. Luciferase assays, RT-qPCR and WB analysis were performed to characterize the KCTD1 function in Hh pathway regulation. MB cell proliferation was investigated through MTS and EdU incorporation assays.

RESULTS

KCTD1 overexpression induces the decrease of HDAC1 protein levels in HEK293T cells and MB cell line, repressing Gli1 activity. As KCTD15, KCTD1 is unable to directly bind HDAC1. KCTD1 bind KCASH2 and KCTD15 enhancing KCASH2 protein stability and improves its inhibitory effect in Hh dependent MB cells. Finally, KCTD1 overexpression impairs MB cell proliferation and leads to both a reduced Hh-dependent proliferation of MB cells.

CONCLUSIONS

Our data suggest an interplay between KCTD1 and KCTD15 in the Hh pathway modulation. We identified a new mechanism involved in fine tuning of the Hh pathway, which exploit the modulation of KCASH2 protein stability, acting at the post-transcriptional level. This mechanism of regulation underlines the relevance of a proteomic approach in the search for potential tumorigenic alterations.

P153

EXTRACELLULAR VESICLES FROM LEPTIN-TREATED MCF-7 BREAST CANCER CELLS SHOW AN ENRICHMENT IN PROTEINS INVOLVED IN ENERGETIC METABOLISM

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BACKGROUND-AIM

Extracellular vesicles (EVs), membrane enclosed nanosized particles, have been reported as crucial mediator in cell-to-cell communication in breast cancer (BC). Recently, we identified leptin, an adipokine which circulating levels are elevated in obesity, as an inducer of EV biogenesis and release in BC cells. Here, we investigated the identification of a specific leptin-induced EV proteomic signature in attempt to find molecular effectors associated with BC progression.

METHODS

Ultracentrifugation method was used to isolate EVs from conditioned media of ER-α positive MCF-7 BC cells; Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and Immunoblotting assay were performed to characterize EVs. Liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS), was used to obtain EV proteomic profile; Gene set enrichment analysis (GSEA) was performed to explore Gene Ontology categories enriched within the up-regulated proteins; Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analysis was used to predict protein-protein interaction.

RESULTS

LC-MS/MS analysis identified 1975 proteins among which 274 were up-regulated (FC>1.5) in EVs from leptin-treated cells vs the untreated ones. GSEA of the entire up-regulated protein list, showed several significantly enriched biologic processes mainly involved in energy production and mitochondrial metabolism, including “generation of precursor metabolites and energy” (GO:0006091), “fatty acid metabolic process” (GO:0006631), “mitochondrial respiratory chain” (GO:0033108), “mitochondrial gene expression” (GO:0140053) and “mitochondrial transport” (GO:0006839). STRING analysis highlighted Cytochrome c-1, NADH:ubiquinone oxidoreductase core subunit V2, and NADH:ubiquinone oxidoreductase subunit AB1, important subunits of mitochondrial complexes and enhancer of mitochondrial metabolism, as the most interactive proteins.

CONCLUSIONS

In BC cells, Leptin could induce the release of EVs enriched in specific proteins mainly involved in mitochondrial metabolism. Understanding the metabolic mechanisms mediated by EVs from leptin-exposed BC cells may provide important clues to develop novel therapeutic approaches for treatment of BC, especially in obesity setting.

P154

UNRAVELING ERK5 CYTO-NUCLEAR SHUTTLING USING IVERMECTIN AND A NOVEL APPROACH FOR SINGLE MOLECULE TRACKING

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BACKGROUND-AIM

The extracellular signal-regulated kinase 5 (ERK5) is emerging as a target for cancer treatment. ERK5 pro-proliferative activities are linked to its presence in the nucleus. ERK5 nuclear translocation is controlled by unexplored mechanisms. We focused on the elucidation of this event using single molecule tracking and molecular biology approaches with the aim to identify compounds able to prevent ERK5 nuclear shuttling and new strategies for cancer treatment.

METHODS

To achieve single ERK5 tracking in living cells, we used Super-Resolution microscopy. HeLa cells were transfected with a vector for ERK5-HaloTag, alone or with a vector for the ERK5 activator MEK5. The cell-permeable photoactivatable dye JaneliaFluor646, able to recognise HaloTag, was used as detection technique. As a complementary approach, HeLa cells, transfected with ERK5 and MEK5, were treated with the alpha/beta importin inhibitor Ivermectin (IVM) and HEK293T cells were transfected with anti-importinB1 si-RNA. MTT assay was performed in A375 and Sk-Mel-5 cells treated with IVM in combination with the ERK5-i AX-15836.

RESULTS

Our results revealed that the HaloTag technology provides the JF646 selective binding to ERK5, and STORM microscopy allows us to collect the signal of individual chromophores. Our data showed that in ERK5-transfected cells ERK5 is mainly localized in the cytoplasm and it moves in the nucleus upon MEK5 overexpression. ERK5 amount in the nuclear fraction of lysates from IVM treated-cells or from anti-impB1-si-RNA transfected cells is lower compared to control, confirming that alpha/beta importins mediate ERK5 nuclear transport. Finally, we found that AX-15836, an ERK5 inhibitor that has been reported to induce ERK5 nuclear translocation with a paradoxical activation, reduced melanoma cell proliferation only in combination with IVM.

CONCLUSIONS

The HaloTag-JF646 method has been proven effective for single ERK5 molecule localization with nanometre accuracy providing a novel approach to evaluate how ERK5 moves to the nucleus. The actors involved in these processes could be identify as novel targets for ERK5 inhibition, and therefore for a possible anticancer therapy. The combination treatment with IVM and AX-15836 reduced melanoma cell proliferation, suggesting the use of AX-15836 as anticancer agent.

P155

DEVELOPMENT OF A TEST FOR THE EVALUATION OF CELLULAR IMMUNE RESPONSES FOLLOWING SARS-COV-2 VACCINATION

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BACKGROUND-AIM

Vaccination against SARS-CoV-2 protects more than 90% of individuals from severe COVID-19 by inducing Spike-specific antibodies and T cells. To date, however, there is no clear definition of antibody and / or T cell levels necessary to confer such protection. To date, empirical data are still limited for the T-cell response due to the technical complexity of SARS-CoV-2 T-cell measurements. The aim of this study is the development of a test for the measurement of Spike-T cell frequencies in vaccinated people, which would allow the profiling of immunodominant peptides correlated with HLA-restriction.

METHODS

Our test is based on ELISpot-IFNγ assay using originally designed stimulating peptides from Spike protein by HLA-peptide binding prediction analysis. PBMC samples were isolated from 15 healthy donor by density-gradient sedimentation after the first and second dose of the SARS-CoV-2 mRNA vaccination. Anti-Spike IgG was assessed by Diasorin Trimeric S-IgG assay. HLA typing was performed by DNA-SSP PCR.

RESULTS

We identified 38 peptides that were predicted to bind the more prominent HLA class I and II alleles. T cells reactive to peptide stimulation were detectable in 70% and 100% of vaccinees after the first and second dose, respectively. The mean frequency of reactive T cells was 28.6 SFU/x106 (CI95% 14.4–42.9 SFU/x106) after the first and 128.5 (CI95% 79.5-177.5 SFU/x106, $p<0.005$) after the second dose. We did not observe any correlation between the frequency of reactive T cells and serum anti-Spike IgG levels. The ELISpot SFU in response to peptide pools from S1 or S2 subdomains showed responses to either S1 or S1 at the first dose samples, while second dose samples had positive responses to both S1 and S2. The small samples size did not allow for any significant association between single peptide response and HLA alleles.

CONCLUSIONS

Our panel of peptides derived from the viral Spike protein was able to reveal a T-cell response in 100% of the cases. The extension of the study to a greater number of cases will allow to highlight possible associations between cellular response and HLA haplotype, helping to clarify any genetic factors capable of influencing the development of protective immunity against SARS-CoV-2 in vaccinated subjects.

P156

NANOPLASTICS & HEALTH: TAKING A PICTURE OF BONE CELLS.

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BACKGROUND-AIM

We live in a plastic world. Plastic waste deeply affects not only our environment but also our health entering the food chain. Only a few studies tested nanoplastic (NP) effects on human health. NP toxic actions are, in part, mediated by oxidative stress (OS) that, among its effects, can influence bone remodeling and epigenome. Epigenetic regulation mechanisms mediate the genome response to environmental stimuli.
My hypothesis is: "Could NPs influence skeleton remodeling through OS?". I have tested my hypothesis on bone physiological environments to take a full-resolution picture.

METHODS

Murine bone cell cultures (MC3T3-E1 preosteoblasts, MLOY4 osteocyte-like cells, and RAW264.7 pre-osteoclasts) were used to test the NPs detrimental effects.
The above cells were treated with increasing NPs concentrations (polystyrene NPs, diameter 50 nm, ranging from 0.1 to 200 μg/ml, exposure from 24 to 72 hours) and tested for cell viability (by MTT assay), ROS level (by DCF) and apoptosis (fluorimetric assay). Cellular morphology and NPs localization were also assessed. We have analyzed the migration capability of MC3T3-E1 cells. Lastly, we have analyzed the transcriptomic profile of MC3T3-E1 and MLOY4 cells.

RESULTS

NPs (from 100 μg/ml, 48 hours exposure) affect cell viability and induce ROS production (both oxygen and nitrogen reactive species) in all cells lines considered. NPs induce apoptosis (by the activation of caspase 3/7) both in preosteoblasts and pre-osteoclasts. NPs affect the preosteoblast migration capability, directly involved in bone remodeling. We found that NPs affect the expression of genes related to inflammatory and osteogenic pathways. In preosteoblasts and osteocytes, NPs reduce the gene expression of HSD11b1, involved in glucocorticoid metabolism. We are now investigating the epigenome contribution of NP detrimental effects in bone cells.

CONCLUSIONS

The environmental impact of NPs is an increasingly worrying problem for human health due to the large-scale growth of plastic production and its resistance to degradation. A better understanding of the impact of NPs on bone cell activities resulting in vivo in impaired bone turnover could give more information on the possible toxicity consequence of NPs on bone mass and the subsequent health problems, such as bone disease.

P157

HEDGEHOG DRIVEN REGULATORY NETWORK SUSTAINS CHEMORESISTANCE AND MESENCHYMAL PHENOTYPE IN COLORECTAL CANCER CELLS

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BACKGROUND-AIM

Colorectal cancer (CRC) is a leading cause of mortality and morbidity. CRC is characterized by frequent development of chemoresistance, achieved by the modulation of signaling pathways that regulate cell survival. Thus, understanding mechanisms related to aggressive features remains one of the most important goal for the development of more efficient strategies.

The deregulation of different oncogenic pathways is involved in the acquisition of aggressive features and chemoresistance. In this context of CRC, we previously showed that the Hedgehog pathway regulates chemoresistance by up-regulating ABC transporters. Together with HH another important pathway involved in stemness features of normal colonic mucosa and is Notch1 pathway.

Recent studies highlight the crosstalk between Notch1 and HH-GLI1 signaling is fundamental in different development contexts.

The aim of our work was to investigate the role of Hedgehog-GLI1 together with Notch1 pathway in CRC resistance to chemotherapy.

METHODS

We performed our experiments in different cellular models derived from two CRC HT29 and HCT116 cell lines. Cells cultured in 2D and 3D (organoids) conditions were treated with 5-Fluoruracil, Notch1 inhibitor (DAPT), and HH-GLI1 inhibitor (GANT-61) and Arsenic Trioxide (ATO) inhibitor of both pathways. Proteins and RNA levels were evaluated.

RESULTS

First, we found that HT29 cells (KRAS mut) and HCT116 (BRAF mut) expressed Notch1 and GLI1 and that the HH and Notch1 inhibitors were able to downregulate the signaling but were not able to induce apoptosis, while the combinatory treatments induce apoptosis.

Moreover, the inhibition of HH-GLI1 and Notch1 was able to induce chemosensitivity to 5-Fluoruracil, by increasing cell death.

The combined inhibition of HH and Notch1 together with 5fu treatment induces differentiation markers axin, e-cadherin and klf4.

In organoids we observed that the combined inhibition of HH-GLI1 and Notch1 together with 5-Fu impaired the protein expression of the Epithelial to Mesenchymal Transition (EMT) marker Vimentin.

CONCLUSIONS

Our data highlight the role of HH- GLI1 pathway together with Notch1 signaling in regulating cell death differentiation and mesenchymal phenotype in CRC cellular models.

P158

NEW INSIGHTS INTO THE ROLE OF THE HECT-E3 LIGASE SMURF2 IN THE MODULATION OF THE HEDGEHOG PATHWAY

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BACKGROUND-AIM

The Hedgehog (Hh) pathway acts through the activation of the GLI family of transcription factors and their target genes, involved in proliferation. The deregulation of the Hh pathway has been widely associated with tumorigenesis, especially in the Hh-dependent medulloblastoma (MB), mainly due to the increase in the transcriptional activity of GLI1, which supports the activation of genes associated with proliferation. Understanding the mechanisms controlling the Hh signaling pathway is essential to identify strategies to counter its deregulation. Among these mechanisms, the role of post-translational modifications, in particular ubiquitination, has become crucial. Therefore, we investigated the potential role of the HECT E3 Ligase SMURF2 in this context.

METHODS

To evaluate SMURF2 effects on GLI1 protein stability we used both HEK293T and D283 cells. Cells were transfected with Smurf2 and Gli1 encoding plasmids. Protein levels were analyzed by Western blot (WB). Hh pathway regulation has been monitored by Luciferase reporter assays and RT-QPCR. Immunoprecipitation (IP) assays were carried out to analyze GLI1 ubiquitination levels and to verify GLI1-SMURF2 interaction.

RESULTS

We observed that increased expression of SMURF2 in vitro can knock down protein levels of endogenous and exogenous GLI1. We also demonstrate a negative effect of SMURF2 on Hh transcriptional activity. Through Co-IP assays we observed an interaction between SMURF2 and GLI1 proteins. The crucial role of HECT E3 Ubiquitin Ligases in protein turnover prompted us to investigate whether the increased expression of SMURF2 could establish a modification of GLI1 ubiquitination status. Indeed, our results suggest that SMURF2 overexpression increases GLI1 ubiquitination levels, and this modification leads to GLI1 degradation. Finally, we have observed that the overexpression of SMURF2 reduces GLI1 protein levels, even in a tumor context, as in the D283 MB cells, suggesting that SMURF2 modulation could be exploited to reduce Hh pathway during MB tumor treatment.

CONCLUSIONS

Our evidence suggests a previously unknown inhibitory role of SMURF2 on GLI1 and show SMURF2 as a new player in the network of regulatory proteins which modulate the Hh pathway. These findings could be exploited for new therapeutic approaches against MB.

P159

LEISHMANIA INFANTUM PARASITES DAMPEN AMYLOID β-INDUCED NEUROTOXICITY AND INFLAMMATION IN MICROGLIA

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BACKGROUND-AIM

Neuroinflammation is a crucial component of Alzheimer's disease (AD), the most common dementing illness. Amyloid β (Aβ) in the brain activates microglia (the macrophages of the CNS), which in turn enhance the neuroinflammatory response through the production of inflammatory cytokines and neurotoxic free radicals. Thus, the prevention of both the exacerbated inflammation and sustained neurotoxicity are highly desired in AD. Leishmania infantum are protozoan parasites that survive within macrophages, where they subvert normal phagocyte functions and innate immune responses. Indeed, our group has demonstrated that L. infantum inhibits the release of pro-inflammatory IL-1β in Aβ-treated human macrophages (Saresella et al., 2020). Yet, whether microglia can phagocytose L. infantum parasites and whether the presence of L. infantum modulates the chronic inflammation by Aβ-activated microglia remains largely unexplored.

METHODS

We used immortalized murine microglia (MMC), and L. infantum parasites stably-transfected to overexpress a chimeric bioluminescent and a red fluorescent fusion protein. MMC were infected with L. infantum at different cell:parasite ratios and treated with LPS, IFN-γ and Aβ as inflammagens. After 24 h, the production of pro-inflammatory cytokines (IL-1β, TNFα and IL-18) and neurotoxic nitric oxide (NO) was assessed by ELISA and the Griess assay, respectively, while microglial viability was studied by the MTT metabolic test. Live cell imaging, Giemsa staining and bioluminescence readouts were used to assess infection parameters.

RESULTS

Our results show that MMC are able to phagocytose L. infantum, whereas the combination of the parasites with a fluorescently-labelled Aβ corroborated the simultaneous co-existence of Aβ and L. infantum in the same microglial cell. Interestingly, the release of pro-inflammatory IL-1β, TNFα and IL-18 along with toxic NO were significantly inhibited by L. infantum in Aβ-stimulated MMC.

CONCLUSIONS

These findings suggest that the parasites and/or parasite-derived compounds might be potentially exploited as immunomodulators against inflammation and neurotoxicity in AD. The exact molecular mechanism must be further investigated.

P160

HIGH ORAL PORPHYROMONAS GINGIVALIS (PG) ABUNDANCE IS ASSOCIATED WITH HDL METABOLISM DYSREGULATION IN BOTH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIC (HEFH) AND HIGH-RISK PATIENTS

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BACKGROUND-AIM

Periodontitis is independently associated with arteriosclerotic vascular disease (ASVD). Low-grade chronic inflammation, promoted by oral and gut dysbiosis may be considered a contributory cause of individual susceptibility to ASCVD observed in the general population and in heterozygous familial hypercholesterolaemia (HeFH). Increased Porphyromonas gingivalis (PG) oral concentrations have been associated with clinical and experimental atherosclerosis. PG induces oxidation and reduction of HDL-C, stimulates atherosclerosis-related gene expression in macrophages and foam cells in the presence of oxLDL. We assessed oral PG abundance and oral health status in very high-risk patients with FH (with/without previous ASCVD) and in patients in secondary ASCVD prevention.

METHODS

36 patients with FH (25% with previous ASCVD) from the LIPIGEN study, 22 non-FH patients in secondary ASCVD prevention, and 31 healthy controls were selected to quantify oral PG abundance through qPCR. All participants underwent a complete oral examinations.

RESULTS

The 3 groups did not differ for smoking, alcohol intake, gingival index, and removable denture. Non-FH patients were older (p=.004) and have a higher BMI (p=.029) than both FH patients (mean difference: 11.5 years and 2.4 kg/m2) and controls (9.8 years and 3.4 kg/m2), and presented a slightly greater proportion of males (p=.104). There was a trend to a higher plaque index (p= .059), a reduced number of teeth (p= .144) and a greater use of mobile prostheses (p= .064) in non-FH patients vs FH patients. PG quantity was also slightly and inversely correlated to number of teeth in non-FH patients (p= .005; rho=−0.298). Oral PG quantity was higher in non-FH patients (p=.047, method of trimmed mean with 0.1 level), particularly compared to controls (p=.054). HDL-C was similar between FH and non-FH groups; LDL-C and TC were higher in FH patients (p<.001), while TGL were lower (p=.016). Oral PG quantity and HDL-C were inversely correlated, although slightly, in both the FH (p= .141; rho=−0.254) and non-FH group (p= .057; rho=−0.254).

CONCLUSIONS

Higher oral PG abundance is present in high-risk patients and is associated to ASCVD events. This association could be explained by the action of PG on HDL metabolism.

P161

LOSS OF KCASH2 CAUSES A CHANGE IN PERMEABILITY OF BOTH BLOOD BRAIN BARRIER AND BLOOD TESTIS BARRIER IN A MOUSE MODEL.

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BACKGROUND-AIM

KCASH2 protein has been described as a negative regulator of the Hedgehog pathway. Trough KCASH2 KO mouse model we highlighted KCASH2 expression in several tissues, suggesting its involvement in multiple biology mechanism. Between others, kcash2 gene is expressed in Cerebellum, Brain, spinal cord and Testis. These tissues undergo a strong control in the composition of their microenvironment and are almost separated from the rest of the organism by the Blood Barrier. This barrier opposes the passage of molecules by means of protein complex that makes up the tight junctions between endothelial cells. Deregulations in Blood Brain barrier and in Blood Spinal Cord Barrier may result in pathological states such as schizophrenia, autism, multiple sclerosis, neuroinflammation and others. Whereas deficiency in Blood Testis Barrier impacts on spermatozoa maturation, leading to potential sterility.

METHODS

To monitor KCASH2 gene expression, we performed enzymatic β-Galactosidase assay in mouse tissue slides or by western blot analysis. To evaluate Blood Barrier permeability, we compared WT and KCASH2 KO mice, and used Evan's Blue dye which is unable to overcome a functional barrier due to its size. To determine the different level of expression of tight junction genes we performed RT-qPCR and western blot analysis on mouse tissues ex vivo.

RESULTS

In vivo intraperitoneal injection of Evan's Blue shows a decrease in Blood Brain barrier permeability in the KCASH2 KO mouse model. Moreover, RT-qPCR analysis in Brain and Spinal Cord tissues highlights the up-regulation of Tight junction complex proteins such as ZO-1, JAM and a particularly strong up-regulation of Claudin 5. On the contrary, in testicles, Claudin 5 expression seem to be down regulated and leads to an increase in the Blood Testis Barrier permeability resulting in a higher number of atypical spermatozoa in KO testis.

CONCLUSIONS

Although the molecular mechanism behind the modulation of tight junction proteins in these context remains unknown, our results suggest a role for the KCASH2 protein in the regulation of Blood Brain Barrier, Blood Spinal Cord Barrier and Blood Testis Barrier. This role may be explained by the known modulation of the Hh pathway, but also by still unknown new function of KCASH2 in the modulation of other protein's stability.

P162

THE IMPORTANCE OF CD44 TRANSCRIPTOME ANALYSIS IN IDENTIFYING NEW TRANSCRIPTIONAL VARIANTS AS MALIGNANT PHENOTYPE'S BIOMARKERS

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BACKGROUND-AIM

CD44 receptor is a transmembrane glycoprotein involved in the recognition of specific ECM ligands, which activate a plethora of signaling pathways. It has a complex transcriptional regulation and diverse splicing variants, such as the CD44 variant 6, which have a role in promoting tumorigenesis as prognostic biomarkers and emerging therapeutic targets. Triple negative breast cancer (TNBC) is a poor prognosis subset of breast adenocarcinomas characterized by the lack of druggable hallmarks. TNBC is a prototypical model of CD44 pro-tumoral activity, being CD44 involved in cancer stemness, cell survival, invasion, and immune escape. However, the regulation of CD44 transcriptome and its influence over TNBC aggressiveness remains elusive. In this study, we dissected the CD44 transcriptome in MDA-MB-231 and MDA-MB-468 TNBC cell lines characterized by a different malignant biology. The MDA-MB-468 forms in vivo poorly differentiated tumors with strong EGFR and high proliferation index, but low tendency to metastasis formation and good therapeutic responses. The MDA-MB-231 line shows a high extracellular matrix invasive potential, prominent metastatic potential, and poorer response to chemotherapy.

METHODS

On the RNA extracted from the two TNBC cell lines, we carried out a CD44 gene-specific retro-transcription followed by PCR enrichment. The cDNA was sequenced through an Oxford Nanopore long-read sequencing and aligned versus the CD44 reference. Following analysis of differentially expressed isoforms, we transfected MDA-MB-468 with a newly identified CD44 isoform (XM_017018585.2) and performed whole transcriptome RNA-sequencing of transfected and control cells.

RESULTS

We identified a different pattern of the CD44 transcriptome between the two TNBC lines, with the XM_017018585.2 showing positive enrichment in MDA-MB-231 cells. The over-expression of XM_017018585.2 in MDA-MB-468 cell line induced the upregulation of transcripts involved in COX2 signaling and the downmodulation of negative EMT regulators.

CONCLUSIONS

The CD44 transcriptome analysis of TNBC cell models revealed new transcriptional variants with potential significance in the modulation of the malignant phenotype.

P163

EFFECT OF THE INDUCTION OF CHRONIC STRESS ON CELLULAR MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND-AIM

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects the upper and lower motor neurons. The hallmark of ALS is the presence of proteinaceous inclusions made of TDP-43 formed through prion-like misfolding, a common process in neurodegenerative diseases. TDP-43 is a nuclear protein that localizes in the cytosol upon acute stress insults. Little is known about TDP-43 behaviour upon milder and prolonged insults, a condition closer to pathology compared to acute stress. Here we aim to elucidate the role of TDP-43 aggregation upon induction of different paradigms of chronic stress in neuronal cell lines stably transfected to overexpress mutant TDP-43 variants

METHODS

Paradigms of chronic stress were applied to neuronal SH-SY5Y cultures for 48 hours and tested for cell viability by MTS assay. Stressors tested included Serum deprivation (Sd, 0,1% FBS), Sodium Arsenite (Ars), Paraquat (PQ), Sorbitol (Sorb) and Lactate (Lact). Stably transfected SH-SY5Y cells were produced to overexpress Myc-tagged TDP-43 variants Q331K and the 35kDa truncated form T86 upon induction with doxycycline. Western Blot (WB) analyses were conducted to assess the expression of TDP-43 under acute and chronic stressful conditions. TDP-43 localization after stress induction was observed by immunofluorescence (IF) and thioflavin-T (ThT) staining

RESULTS

Sub-lethal concentrations of Sd, Ars, PQ and Sorb were identified by MTS assay. The end-point of the pathological phenotype was assessed by WB under acute stress conditions, where TDP-43 is mostly found in the insoluble fraction. Chronic treatment revealed a similar pattern in terms of TDP-43 solubility. IF experiments revealed different aggregation patterns of TDP-43, which may imply that different TDP-43 aggregation pathway may be involved in response to prolonged stress

CONCLUSIONS

The results thus far gathered indicate that: (1) TDP-43 overexpressing cells sustain prolonged exposure to Sd, Ars, PQ and Sorb; (2) stress paradigms cause TDP-43 mobilization and produce an increase of insoluble TDP-43 species (3) ThT staining can be conducted to observe TDP-43 related amyloid structures. Unraveling TDP-43 aggregation patterns during chronic stress may help to elucidate the onset and progression of ALS and related pathologies

P164

SELECTIVE SORTING INTO EXTRACELLULAR VESICLES OF DIFFERENT SMALL RNA SPECIES DURING ENDOTHELIAL CELL SENESCENCE AND HIGH-GLUCOSE STRESS

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BACKGROUND-AIM

The active sorting of different families of small RNAs into extracellular vesicles (EVs) is a novel, yet incompletely understood, mechanism of intercellular communication. Accumulating evidence demonstrated that various cellular stressors affect EV biogenesis and their cargo. Here, we aimed to explore changes in the EV small RNA secretome by taking advantage of previously established in vitro models of endothelial cell replicative senescence and high glucose exposure.

METHODS

Small EVs (sEVs) were purified from conditioned media of non-senescent (CON) and senescent (SEN) HUVECs cultured for 7 days under normal (5.5 mM, NG) or high (25 mM, HG) glucose concentrations. Small RNA-seq was performed on cells and sEVs and the expression of different small RNA (<70 bp) species, including non-coding (nc)RNAs originating from genomic repeats, was assessed.

RESULTS

SEN cells exhibited a higher content of ncRNA compared to CON cells (CON, 63±2%; SEN, 69±3% of mapped reads). HG treatment resulted in a further increase in the relative abundance of ncRNA species in both conditions (CON-HG, 82±1%; SEN-HG, 80±1%). SEN cells released a greater number of sEVs compared to CON cells, and HG induced a proportional increase. Both senescence and HG were associated with an increased sEV content of ncRNAs (CON, 5±5%; SEN, 53±5%; CON-HG, 82±1%; SEN-HG, 80±1%). Analysis of ncRNA classes revealed an enrichment in rRNA fragments in sEVs compared to their parent cells (CON, 31±2%; CON sEVs, 95±3% of ncRNA reads), which was further increased by HG (CON-HG sEVs, 99±1%; SEN-HG sEVs, 97±2%). In cells, the amount of small RNAs mapping to repeated sequences (about 25%) was comparable among conditions, with rRNAs (54%) and tRNAs (32%) being among the most represented species. sEVs from CON cells displayed an enrichment in small RNAs transcribed from transposable elements (18±2%), which was blunted in SEN (1±1%) and HG (3±1%) sEVs, where a prevalence of rRNA was observed.

CONCLUSIONS

Replicative senescence and HG induce notable alterations of the EV small RNA profile, which could provide insights on the intercellular spreading of the senescent phenotype and a useful tool in the development of specific biomarkers of cellular senescence and metabolic stress.

P165

GEMCITABINE MODULATES CELLULAR AND EXOSOMAL PDL-1 IN PANCREATIC CANCER

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BACKGROUND-AIM

Pancreatic cancer (PC) is refractory to available immune checkpoint blockade and it is characterized by loss of tumor-infiltrating effector lymphocytes and by the presence of an immunosuppressive tumor microenvironment. The PD-1/PD-L1 axis and other immune checkpoints inhibit antitumor immune response and PD-L1 is highly expressed by several tumor cells and their exosomes. Our study aimed at investigating whether the PC first-line drug gemcitabine modulates immune checkpoint proteins expressed by PC cell lines and by their extracellular vesicle (EVs) affecting immunosuppressive potential of these cells.

METHODS

PC cell lines AsPC-1, Capan-2 and BxPC-3 were treated with gemcitabine at different dosages (0.1 μM or 1μM) or with vehicle. EVs detection was carried out in cell conditioned media as LCD+/phalloidin- events by a patented protocol using polychromatic flow cytometry and volumetric count. Exosomes were isolated from cell conditioned media by sequential centrifugations and ultracentrifugations. The effects of gemcitabine on PDL-1 expression at the cell surface and in exosomes derived from PC cell lines were analyzed by flow cytometry and by western blot.

RESULTS

We observed that AsPC-1, Capan-2 and BxPC-3 cells expressed both surface and cytoplasmic PDL-1. Gemcitabine treatments increased surface PDL-1 in AsPC-1 and BxPC-3 at both dosages. These data were corroborated by western blot analysis of PDL-1 expression. Even at low dosages, gemcitabine induced an increase of EVs concentration in conditioned media of Capan-2 and BxPC-3. The drug induced PDL-1 expression in exosomes isolated from conditioned media of AsPC-1 and BxPC-3, whereas in Capan-2 high exosomal PDL-1 expression was observed already in basal conditions.

CONCLUSIONS

PDL-1 expression both in PC cell lines and in their secreted exosomes was increased by gemcitabine. The drug increased also EVs concentration in conditioned media of PC cell lines. We are currently analyzing by a shotgun proteomic approach whether exosomes derived from untreated or gemcitabine-treated PC cells affect protein expression in CD3+ T.

This study was supported by PRIN 2017 EKMFTN_005

P166

RESVERATROL IMPROVES PREP1-INDUCED ENDOTHELIAL DYSFUNCTION IN AGED MICE AND IN ENDOTHELIAL CELLS.

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BACKGROUND-AIM

Aging is associated to a progressive endothelial dysfunction caused by an increase of inflammatory cytokines production and, a reduction of nitric oxide (NO) bioavailability. Resveratrol, by regulating different transcription factors, has been shown to have numerous health benefits. Prep1 is a homeodomain transcription factor which increases in the aorta of aged mice and in senescent vascular smooth muscle cells (VSMCs) and is involved in the neointimal formation by reducing VSMCs apoptosis. In this study, we have evaluated the role of Prep1 in the aging-related endothelial dysfunction.

METHODS

Proteins and mRNA levels from 6- and 18-months old WT and Prep1 hypomorphic heterozygous mice ($Prep1^{i/+}$) mice or mouse aortic endothelial cells (MAEC) transfected with Prep1 and treated with IL6 and resveratrol were analyzed by Western blot and Real-Time RT-PCR. Nitric Oxide was detected using a Nitrite/Nitrate Assay colorimetric kit.

RESULTS

18-month-old $Prep1^{i/+}$, expressing low levels of protein, showed a reduction of oxidative stress biomarkers and proinflammatory cytokine levels. NO release and $eNOS^{Ser1177}$ phosphorylation, which activates NO production, increased by 20% and 30% compared to the WT littermates. In parallel, phosphorylation on Threonine 495 residue, which inhibits eNOS function, was 40% lower in $Prep1^{i/+}$ mice compared to the WT animals. Upstream eNOS regulators, as PKCα and PKCδ, were reduced in mice lacking Prep1, while Akt/PKB did not change. Consistent with these results, Prep1 overexpressing MAECs featured an increase of oxidative stress biomarkers while eNOS-mediated NO production decreased. Interestingly, inhibition of PKCα and PKCδ by staurosporine restored eNOS function. Treatment of MAEC cells with resveratrol prevents IL6 induced Prep1 proinflammatory cytokines.

CONCLUSIONS

Our results indicate that Prep1 impairs endothelial function during the aging and is modulated by resveratrol, suggesting Prep1 as a novel possible target in the age-related vascular diseases.

P167

PREDICTIVE POTENTIAL OF THE UPPER AIRWAY MICROBIOME COMPOSITION IN HEMATOLOGICAL PATIENTS AT RISK FOR INVASIVE FUNGAL INFECTIONS

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BACKGROUND-AIM

Hematological patients are at major risk for developing invasive fungal infections (IFIs), opportunistic diseases that cause significant morbidity and mortality, and the possibility of stratifying patients based on actual risk for IFI would be of fundamental importance to individualize therapy. It is now clear that the airway microbiome is emerging as an important player in tissue physiology and in protection against colonization by respiratory pathogens including fungi.

METHODS

Based on these premises, we have designed a multicenter, prospective, observational study termed SNIF (Survey of Nasal InFection) in which hematological patients were recruited and their nasal and oropharyngeal swabs collected over a 6-month period for microbiome characterization. Patients were stratified for the risk of IFI according to clinical parameters. The microbiome signatures were determined by high dimensional class comparisons using linear discriminant analysis of effect size (LEfSe), and the functional potential predicted by using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)2.

RESULTS

A total of 212 patients diagnosed with hematological malignancies were enrolled, and 1,000 nasal and oropharyngeal samples analyzed. Consistent with previous findings in volunteers, the noses and the oropharynges were found to harbor distinct microbial communities as measured by diversity indexes and differential abundance testing methods. Further characterization of the nasal and oropharyngeal microbiota following stratification based on the risk for IFI, revealed low diversity dysbiosis in high-risk (HR) compared to low-risk (LR) samples, with the loss of common taxa associated with health and the emergence of potential pathogenic bacteria. Prediction of functional potential revealed the presence of metabolic pathways with significant different abundance in the LR and HR groups.

CONCLUSIONS

The results indicate significant differences in microbial composition between nose and oropharynx as well as between patients at different risk for IFI. Whether and how the compositional and functional changes impact on the different risks for IFI is under investigation as well as how this information could translate into clinical practice.

P168

ANTIBODY TITERS AFTER BNT162B2 MRNA VACCINE ADMINISTRATION IN PATIENTS UNDERGOING IMMUNOMODULANT THERAPIES, MEASURED WITH THREE DIFFERENT SEROLOGICAL IMMUNOASSAYS

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BACKGROUND-AIM

The aim of the study is to evaluate the antibody response to BNT162b2 vaccine administration in subjects affected by psoriasis and/or psoriatic arthritis treated employing different immunosuppressive/immunomodulant schemes.

METHODS

This study recruited 45 subjects, without history of SARS-CoV-2 infection, following BNT162b2 vaccination. Patients were grouped into 4 different categories according to their undergoing drug-therapy: anti-TNF (n=9), anti IL-17 (n=13), anti IL-23 (n=13) and topic drugs (n=11). Serum of the participants was collected at T0 (before vaccination), T1 (15 days after the 1st dose) and T2 (15 days after the 2nd dose) and tested to evaluate the quantitative serologic neutralizing antibody response, with three different serological immunoassays: ADVIA Centaur SARS-CoV-2 IgG (Siemens Healthineers), Elecsys Anti-SARS-CoV-2 S (Roche Diagnostic) and LIASON SARS-CoV-2 TrimericS IgG (Diasorin).

RESULTS

Vaccine-induced neutralizing antibodies against SARS-CoV-2 were detected in every subject tested, with an excellent agreement between the three type of commercial assays, where the Spearman rho correlation coefficient was for all the assays > 0.95 and the $p < 0.0001$.
Next, we tested by two-way ANOVA the independent contribution of time and drug therapy to antibody production. We observed that no drug treatment significantly interfered with response to vaccination ($p=0.5$, $p=0.1$, $p=0.6$ for Siemens, Roche and Liason, respectively). Conversely, a highly significant association of antibody titer with time from vaccination was found ($p < 0.0001$). However, according to Roche and Diasorin assays, patients treated with anti-TNF therapy did not show a significant elevation of antibody titer at T2.

CONCLUSIONS

Pfitzer BioNTech vaccine-induced antibody response seems to be significant in patients undergoing immunosuppressive/immunomodulant therapy and not related to the category of drug treatment, showing a higher increase in antibody levels following the second dose, confirming the importance of completing the usual recommended vaccination scheme (2-dose administration).

P169

THE ROLE OF NOTCH2 IN THE EXTRACELLULAR VESICLES-MEDIATED ANGIOGENESIS AND OSTEOCLASTOGENESIS IN MULTIPLE MYELOMA

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BACKGROUND-AIM

Multiple myeloma (MM) is an incurable hematological neoplasm mainly due to the interaction with a highly supportive bone marrow (BM) niche. The aberrant expression of the NOTCH2 oncogene in MM cells contributes to MM pathological communication with the BM cells leading to tumor angiogenesis and osteoclastogenesis, two key steps in MM progression.

Recently, extracellular vesicles (EV) shed by tumor cells have been identified as key players in the communication between tumor and microenvironment. This work explores the tumorigenic effect of MM-derived EV (MM-EV) and the role of NOTCH2 in EV-mediated communication.

METHODS

Size and concentration of MM-EV from MM cell lines, RPMI8226 and OPM2, were characterized by nanoparticle tracking analysis along with morphology by transmission electron microscopy and MM-EV uptake by target endothelial cells and osteoclasts (fluorescent microscopy and flow cytometry). MM-EV content and ability to transfer NOTCH2 was assessed by Western blot analysis.

To assess the role of NOTCH2 in EV-mediated communication, we used EV from MM cells knocked down for Notch2 (MMN2KD-EV). The difference in MM-EV and MMN2KD-EV size and concentration was assessed together their ability to activate Notch signaling in recipient cells by two reporter assays performed on HeLa cells and on a Notch-reporter Tg(T2KTp1bglob:hmgb1-mCherry)jh transgenic zebrafish embryo. The osteoclastogenic ability of MM-EV and MMN2KD-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

MM-EV carry and transfer NOTCH2 in a paracrine way, inducing NOTCH signaling activation in recipient cells. Moreover, MM-EV display angiogenic and osteoclastogenic potential depending on the presence of NOTCH2. NOTCH signaling blockade by γ-Secretase inhibitor can interrupt MM-EV mediated pathological communication resulting in angiogenesis and osteoclastogenesis.

CONCLUSIONS

MM-EV promote the progression of MM by promoting tumor angiogenesis and osteoclastogenesis. NOTCH2 plays a key role in EV-mediated communication in the BM microenvironment. Thereby targeting NOTCH activation may represent a suitable strategy to hamper the pro-tumorigenic activity of MM-EV.

P170

VULNERABLE AND NON-VULNERABLE ATHEROSCLEROTIC PLAQUES: ROLE OF CYTOKINES AND VIRAL INFECTIONS

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BACKGROUND-AIM

Cardiovascular diseases due to atherosclerotic plaques represent the major cause of death in developed countries. Several studies have reported an association between viral infections and pathogenesis of atherosclerosis. Microbial agents can promote destabilisation of atherosclerotic plaques through a direct pathogen-mediated damage or through systemic effects of inflammatory cytokines. The aim of the study was to examine stable and unstable atherosclerotic plaques for the presence of genomic sequences of viral agents and the expression of inflammatory cytokines. Levels of circulating cytokines were also evaluated.

METHODS

Carotid plaques and plasma samples were collected from 50 patients undergoing carotid endarterectomy. 31/50 (62%) had a vulnerable plaque. Influenza (IV), Epstein-Barr (EBV), Citomegalo (CMV), Herpes Simplex (HSV) viruses were investigated by RT-qPCR in the “core” of the plaque and in an adjacent non plaque area (internal control). Gene expression of TNFα, IL-1ß, IL-6, RANTES (CCL5) and IL-10 was evaluated by RT-qPCR. Plasma levels of TNFα, IL-1ß, IL-6, RANTES (CCL5) and CXCL8 were evaluated by ELISA.

RESULTS

Only the EBV genome was detected in the core of two vulnerable plaques, but not in their respective non plaque adjacent controls. TNFα was significantly more expressed in the core than in the control area both in vulnerable and stable plaques. Pro-inflammatory cytokines IL-1ß, IL-6 and RANTES (CCL5) were more expressed in plaques’ core vs. control only in vulnerable plaques. Patients with vulnerable plaques showed statistically relevant plasma levels of TNFα and IL-6 compared to patients with non-vulnerable plaques.

CONCLUSIONS

Patients with vulnerable plaques have higher levels of both systemic and local inflammatory cytokines, irrespectively of virus exposure. These data confirm a major role of cytokines in the stability of atherosclerotic plaques.

P171

INCREASED BLOOD CONCENTRATION OF EXTRACELLULAR VESICLES DERIVED FROM CANCER STEM CELLS PREDICTS POOR CLINICAL OUTCOMES IN PATIENTS WITH ADVANCED COLORECTAL CANCER

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BACKGROUND-AIM

Extracellular vesicles (EVs) are nanosized membranous structures secreted by cells to regulate intercellular communication. EVs released by cancer cells are valuable carriers of tumor information and have a promising potential as biomarkers in cancer diagnosis, prognostication, and surveillance. In our study, we investigated the predictive and prognostic role of blood circulating EVs expressing cancer stem cell markers in a cohort of advanced colorectal cancer patients.

METHODS

This prospective study enrolled patients with histologically or cytologically confirmed diagnosis of advanced colorectal cancer. Patients were recruited from July 2018 to August 2021. Identification, enumeration, and phenotypic characterization of EVs from whole fresh blood samples were obtained by applying a patented simplified polychromatic flow cytometry protocol based on a combined EV staining with a lipophilic cationic dye (LCD) and phalloidin. EV subtyping was based on positivity to selected cancer stem cell markers, including CD133, EPCAM, CD29 and CD90.

RESULTS

A total of 54 cancer patients (colon cancer [n=33]; rectal cancer [21]) and 48 healthy controls (HCs) were enrolled. Median blood concentration of total circulating EVs was doubled in the cancer population (median EVs/µl= 5264.0; 95 % CI 4123.0-6314.0) compared to HCs (median EVs/µl= 2548.0; 95 % CI 2100.7-3051.4) (p. 0.000003). Median blood concentration of circulating CD133+ EVs was 2.9-fold higher in the cancer population (median EVs/µl= 52.6; 95 % CI 35.0-98.8) compared to HCs (median EVs/µl= 18.4; 95 % CI 11.6-32.2) (p. 0.007). Survival analysis revealed a correlation between high CD133+ EV concentration (>168 EVs/µl) and worse overall survival (HR= 3.17 [95 % CI 1.37-7.35]; p. 0.007). In the treatment-naïve cohort (n=33), increased level of circulating EVs expressing CD133 was associated with lower overall response rate to first line systemic treatment (p. 0.01).

CONCLUSIONS

Overall, our findings suggest a possible correlation between blood circulating CD133+ EVs and clinical outcomes in patients with advanced colorectal cancers.

This study was supported by PRIN 2017EKMFTN_005.

P172

THE CIRCADIAN CONTROL OF TRYPTOPHAN METABOLISM IN INFECTIOUS DISEASES

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BACKGROUND-AIM

Circadian rhythms govern almost every aspect of life on earth. Thanks to the presence of a molecular clock ticking in each cell, organisms from different domains of life are enabled to anticipate environmental changes thereby adapting their behaviour and physiology to the external light-dark cycles. In mammals, a circadian pacemaker in the brain entrains the clocks located in the peripheral tissues, resulting in the circadian rhythmicity of a large array of biological processes, including metabolism, behaviour and immunity. The metabolism of the essential aromatic amino acid tryptophan (Trp) consists in three major pathways, leading to the production of kynurenine (kyn), serotonin and indole derivatives. While the serotonin pathway is intimately linked to the circadian clock through its end product melatonin, little is known about the interactions between the clock system and the kyn pathway, the rate-limiting step being regulated by three enzymes. The enzyme Indoleamine 2,3-dioxygenase 1 (IDO1) in particular has been the subject of intense research for its role in maintaining homeostasis and plasticity of the immune system, and the Trp-IDO1 pathway has been involved in balancing resistance and tolerance to fungal infections.

METHODS

Here we performed in vivo studies to establish whether the Trp metabolic pathway is controlled by the circadian clock and how this regulation influences the immune response to fungal pulmonary infections. Moreover, we performed in vitro studies to dissect the mechanism of circadian regulation of the Trp-IDO1 axis.

RESULTS

We obtained results showing that IDO1 expression and activity are under circadian regulation in multiple tissues. In the lung, this time-dependent activation results in a significant change in the outcome of Aspergillus fumigatus infection, evaluated by assessing degree of colonization, level of inflammation and immunological mechanisms involved.

CONCLUSIONS

We concluded that the circadian clock, through the modulation of Trp metabolism, controls the host response against fungal infections, a result that might be particularly relevant in pathologies associated with aberrant immunity and inflammation, such as cystic fibrosis, characterized by increased predisposition to this type of infection.

P173

DONOR-DERIVED CELL-FREE DNA (DD-CFDNA) FOR ASSESSING CAV DEVELOPMENT IN HEART TRANSPLANTED PATIENTS

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BACKGROUND-AIM

Cardiac Allograft Vasculopathy (CAV) is a leading cause of long-term graft dysfunction and loss after Heart Transplant (HT). The importance of the early highlight of CAV development is a marker of aggressive disease with poor clinical outcome. Despite advancements in imaging strategies, the standard for CAV assessment remains coronary angiography. Donor-derived cell free DNA (dd-cfDNA) has recently been introduced as a novel marker of graft injury in solid organ transplants. Elevations in dd-cfDNA levels may reflect new or progressive CAV development during subclinical inflammation. For this reason, dd-cfDNA could represent a non-invasive method of surveillance for CAV rejection in apparently stable post-transplant patients. Since the emerging importance of circulating biomarkers and the lack of evidences in the field of chronic rejection in heart-transplant, we decided to validate a standardized and reproducible analytical procedure to evaluate the potential of dd-cfDNA as an alternative diagnostic biomarker for CAV.

METHODS

20 blood samples from patients who underwent heart transplant at the ASUFC Cardiac Surgery Unit in the last 10 years, were analyzed by Next Generation Sequencing (NGS) according to the specific protocol described by CareDx Alloseq assay. The cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit (Qiagen) starting from 2mL of plasma. Sequencing was performed on the Illumina Miseq platform (Illumina); data were interpreted with the AlloSure (CareDx) software.

RESULTS

dd-cfDNA was found in 100% of cases. Considering the cut-off proposed in literature (0,12%), high dd-cfDNA levels were found in our cohort of CAV patients.
A significant difference in dd-cfDNA fraction was found between CAV and no-CAV patients (p=0,044); with mean values of 0,30% e 0,09%, respectively.

CONCLUSIONS

With this study, we demonstrated the feasibility of evaluating dd-cfDNA in long-term heart transplanted patients and its possible association with CAV development. The preliminary results obtained with the optimized analytical procedure suggested a potential predictive value of dd-cfDNA in combination with other biomarkers for a better stratification of patients. The definition of "homogeneous" subgroups of patients will allow to better manage their follow up and improve their prognosis.

P174

G-QUADRUPLEX LIGAND BRACO-19 INHIBITS G-QUADRUPLEX MOTIFS AND COMPROMISES ADIPOCYTE DIFFERENTIATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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BACKGROUND-AIM

Mesenchymal stem cells (MSCs) comprise a subpopulation of multipotent adult stem cells able to differentiate into mesodermal cell lineages, i.e. adipocytes, chondrocytes, osteoblasts. Studies reported the relevance of telomerase in MSCs differentiation and its decline associated with a senescent phenotype. G-quadruplex (G4) structures, based on a tetrad of guanines, were detected in promoter regions as well as in telomeres and their formation/stabilization is known to inhibit telomerase activity in cancer cells. To better understand the mechanisms involved in MSCs function, we investigated DNA G4 motif formation during adipocytes differentiation of adipose-derived MSCs.

METHODS

MSCs were isolated from human adipose tissue biopsies (Ad-MSC). Adipocyte differentiation of Ad-MSC was carried out in the presence of the G4 ligand BRACO-19. DNA G4 motifs and cell cycle were analyzed by flow cytometry. Lipid accumulation was quantified by Oil Red O staining. mRNA expression was measured by qPCR. Cell viability was determined by sulphorodamine B assay. Protein levels were quantified by ELISA.

RESULTS

DNA G4 motifs were mainly distributed in S and G2/M phases and detected at higher levels in differentiated adipocytes compared to undifferentiated MSCs. BRACO-19 treatment was accompanied by reduced G4 motif abundance and decreased intracellular accumulation of lipids. PPARG, AP2, leptin, Oct-4, Nanog and TNF-α mRNA levels were also reduced by BRACO-19. No inhibition of cell proliferation and no detection of subG0 phase in the cell cycle was observed. Of note, BRACO-19 reduced VEGF protein secretion, with no difference observed in IL-8 and IL-6 production, in conditioned media from differentiated adipocytes.

CONCLUSIONS

G4 motif formation increases in Ad-MSC differentiated adipocytes. BRACO-19 G4 ligand compromises the differentiation process likely retaining MSCs in a steady un-differentiated phenotype and reduce the production of pro-inflammatory/pro-angiogenic molecules. This phenomenon might be of interest in pathological conditions requiring MSC differentiation and function, including obesity, type 2 diabetes and cancer progression.

P175

CIRCADIAN REGULATION OF THE HOST RESPONSE TO PULMONARY INFECTIONS IN CYSTIC FIBROSIS

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BACKGROUND-AIM

All living organisms have intrinsic physiological rhythms synchronized with the light-dark cycle. This type of rhythmicity is controlled by the circadian clock, an autonomous biochemical oscillator coordinated by a complex clock gene network. Perturbations of these fine-tuned mechanisms are invariably linked to the development of pathological conditions, from depression to metabolic, cardiovascular and inflammatory disorders. In particular, the circadian clock controls many immune functions, including antimicrobial host defense, and its dysregulation is associated to increased inflammation and risk of infection, particularly in the lung.
Cystic fibrosis (CF) is a genetic multisystemic disorder affecting more than 70,000 people worldwide, mainly characterized by exacerbated airway inflammation and tissue damage, associated to persistent infections responsible for declining pulmonary function. CF patients experience altered sleep-wake cycles, aberrant immune responses and dysregulated metabolism that could be ultimately traced back to perturbations of the circadian rhythms. Since the relationship between CF and circadian clock has remained unexplored, herein we investigate how they are connected in the context of susceptibility to infections.

METHODS

We performed in vitro and vivo studies in order to identify the presence of circadian alterations in CF, by using human bronchial epithelial cells from CF patients and a CF murine model. Moreover, we evaluated time-changing inflammatory and immune responses to pulmonary infection with Aspergillus fumigatus, by assessing the degree of colonization, the level of inflammation and immunological mechanisms involved.

RESULTS

We observed a diurnal time-dependent control of A. fumigatus infection, and an altered rhythm in CF mice and cells. Moreover, we observed that the circadian regulation of the Trp metabolic pathway, involved in balancing resistance and tolerance to fungal infections, plays a central role in the observed day-night differences in the immune response to fungal infection.

CONCLUSIONS

A circadian clock dysregulation in CF functionally affects the host response to pathogens, ultimately leading to aberrant inflammation, a result that may guide novel therapeutic strategies in antimicrobial and antiinflammatory treatments of patients.

P176

STILBENE-BASED COMPOUNDS STRUCTURALLY RELATED TO RESVERATROL EXERT ANTIPROLIFERATIVE ACTIVITIES ON PANCREATIC CANCER CELL LINES

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BACKGROUND-AIM

Pancreatic cancer (PC) is one of the most lethal and chemoresistant malignancies worldwide. Thus, novel and more effective drugs to be employed in PC therapy are needed. Resveratrol (RSV) is a natural polyphenolic phytoalexin that shows antimicrobial, antioxidant, anti-inflammatory and neuroprotective activities in cells, together with chemopreventive and anticancer properties in different tumor models. In this study, we tested the antiproliferative effects of previously synthesized stilbenols, structurally related to resveratrol, on three distinct PC cell lines.

METHODS

MTT assays were performed to evaluate the effects of stilbenols on the viability of three PC cell lines, namely AsPC-1, Capan-2 and BxPC-3, and of normal HFF-1 fibroblast cells. The impact of the most active compounds on PC self-renewal capacity was evaluated by using clonogenic assays. Flow cytometry was used to assess the effects of such compounds on cell cycle and apoptosis. A trypan blue exclusion test was employed to dissect the effect of these compounds on PC cell proliferation.

RESULTS

In the series of synthesized stilbenols, several molecules markedly inhibited PC cell viability, showing IC_{50} values lower than those obtained with reference compounds RSV and 4-hydroxystilbene (4-HSLB) in the same PC cell lines. The most potent compounds in reducing PC cell viability were selected to further explore their antiproliferative activities. Specifically, a compound of the series consistently affected both PC cell line viability and clonogenicity, with enhanced effects as compared to RSV. Notably, both this compound and RSV showed negligible toxicity on normal HFF-1 fibroblast cells. Moreover, they induced PC cell apoptosis with comparable effects and interfered with cell cycle progression, displaying slight differences among the tested PC cell lines.

CONCLUSIONS

Overall, all synthesized stilbenols impacted on PC cell viability in a dose-dependent fashion, showing a variability that appears structure- and cell-dependent. One compound emerged as the most potent derivative in the series, with comparable, or even improved antiproliferative activity as compared to RSV. In the perspective of clinical translation, we are further testing the ability of this novel compound in modulating key processes in cancer cell biology.

P177

PGLHV, A PUTATIVELY NEW CYTOMEGALOVIRUS-RELATED VIRUS IMPLICATED IN PARAGANGLIOMA*

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BACKGROUND-AIM

Our data suggest that paragangliomas (PGLs) arise through a deregulated developmental program highjacked by a human cytomegalovirus-like herpesvirus, preliminarily designated paraganglioma associated herpes virus (PGLHV) (Verginelli F, et al, 2018 and unpublished data). This potentially tumorigenic infection could be facilitated by germline and/or somatic alterations in metabolic genes. We detected PGLHV in virtually all PGLs and PGL-derived xenografts (PDXs) analyzed with several methods, but PCR amplifications were poorly reproducible. Here, we report the isolation of PGLHV virions and the preliminary characterization of the viral genome.

METHODS

PGLHV virions, obtained from PGL/PDX tissues using Rous's protocol (Rous P, 1911) coupled with PEG precipitation, were characterized by FACS and TEM. RNA from PGL and PDX tissues and DNA from virions were used to isolate PGLHV genomes using either a modified targeted RNA-seq (Agilent SureSelect, 44.0000 custom HCMV probes) for Illumina sequencing or Oxford Nanopore Technology (ONT) with whole genome amplification for ONT library preparation, followed by metagenomics analysis. Sequence homology was verified relative to known HCMV strains using bioinformatic tools.

RESULTS

Our initial assumption was that the virus was an HCMV variant. Therefore, we analyzed by SureSelect-targeted Illumina RNA seq X PGLs (PTJ78T, PTJ146T, PV158T) and a derived PDX (PTJ146PDX). Metagenomics analysis attributed to the human beta herpesvirus 5 (HCMV) 62%, 47% and 8% of the total reads obtained for the 3 PGLs, respectively. The viral reads for PTJ146PDX accounted for 1% of the total, of which 8% attributed to HCMV. The total aligned length relative to HCMV (Merlin strain) ranged from 1.45% to 16.76% (39.509/235.646 reads). ONT sequencing of particles isolated from 2 PGLs (PV158T and PTJ153T) confirmed a relationship to HCMV.

CONCLUSIONS

We partially resolved the genome sequence of PGLHV, demonstrating that this putatively new virus shares extensive regions of sequence homology to HCMV, in agreement with the HCMV-like structure of the PGLHV particles. However, PGLHV contains regions that are substantially different from those of HCMV, strongly suggesting that it represents a new member of the Betaherpesvirinae.

*Acknowledgement: work supported by AIRC IG 24501

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TARGETING HEPARAN SULFATE BIOSYNTHESIS TO OVERCOME DRUG RESISTANCE IN OVARIAN CANCER

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BACKGROUND-AIM

The chemotherapeutic agents most commonly used in the treatment of Epithelial Ovarian Cancer (EOC) are platinum agents. The major challenge in the clinical management of EOC patients is the development of platinum resistance. Based on the response to platinum-containing drugs, EOC patients are usually classified as refractory, resistant, and sensitive. The architecture and remodelling of the extracellular matrix play an important role in the development and progression of cancer. The ECM profile is relatively unexplored in EOCs, and data on the involvement of the ECM in platinum resistance are still fragmentary.

METHODS

Copy Number Alterations (CNAs) reflected by altered mRNA expression were analysed in EOC patients from the TCGA dataset and stratified as follows: 14 refractory, 59 resistant, and 107 sensitive patients. The PathScore algorithm was used to identify pathways enriched by changes associated with the three different groups of treatment responses. Exostosin-1 (EXT1) protein levels were assessed by immunohistochemistry on a tissue micro-array of 10 refractory, 15 resistant and 25 sensitive patients. Ovarian cancer cells, COV318 and SKOV3, were transfected with a vector expressing the EXT1 protein to study the deposition and distribution of Heparan Sulfate (HS) and determine how it affects signal transduction and drug sensitivity.

RESULTS

The gene encoding EXT1, an enzyme involved in the extension of the saccharide chain of heparan sulfate proteoglycans (HSPGs), was frequently amplified in refractory compared to platinum-sensitive patients ($p < 0.01$) catalogued in TCGA. Immunohistochemical analysis of EXT1 revealed increased protein levels in 60% of refractory patients compared to 24% of sensitive patients ($p < 0.05$). EXT1 overexpressing cells synthesized an increased amount of HSPGs and showed altered motility and response to platinum-based drugs.

CONCLUSIONS

Our results suggest that the biosynthesis of HS affects ovarian cancer cell characteristics and may play an important role in the development of recurrence in refractory patients. Identification of molecular alterations involved in the development of platinum resistance could help in the selection of women who may be resistant, sparing them ineffective treatments and unnecessary toxic effects.

P179

UNCONVENTIONAL DROPLET DIGITAL PCR ASSAYS FOR SMN1/SMN2 ASSIGNMENT OF PATHOGENIC POINT MUTATIONS

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BACKGROUND-AIM

Almost 4% of spinal muscular atrophy affected patients (SMA) carries the deletion of one copy of the Survival Motor Neuron gene 1 (SMN1) and e deleterious point mutation on the other copy of the gene. Correct assignment of the point mutations to the disease causative gene is not a rapid procedure due to the existence of the twin Survival Motor Neuron gene 2 (SMN2) not involved in the onset of the disease. Transcripts sequence, cloning and clones screening are the standard procedures to establish the localization of the SNP. ddPCR is a new technology able to absolute quantify the number of target nucleic acids using binomial Poisson statistics. Due to its high sensitivity, ddPCR is often employed in pathological conditions. The aim of this work is to address the use of ddPCR technology for an accurate and rapid assignment of the deleterious point mutation in exon 3 to SMN1 or SMN2 responsible of SMA pathological phenotype onset and severity.

METHODS

In this study a SMA type 3A patient, a SMA carrier and a healthy control were enrolled. For the ddPCR assay, RNA was extracted from PBMC and then reverse-transcribed into cDNA. SNP analysis was conducted in two independent reactions for the exon 3-wild type and mutant alleles. Droplet generation was performed by the QX200. Data were computed by QuantaSoft.

RESULTS

A ddPCR specific assay was developed for the detection and assignment of the deleterious point mutation in exon 3. As we expected, the healthy subject and the healthy carrier exhibited higher quantity of SMN1 transcript (90% and 77%, respectively) than SMN2. Conversely, the SMA patient was characterized by low levels of mutant SMN1 transcript (6,3%). Co-segregation study showed that both in the healthy carrier and in the SMA patient the exon 3 mutation was carried by SMN1. This observation could suggest that the point mutation has a deleterious effect on the transcription efficiency.

CONCLUSIONS

The method we developed relay on ddPCR technology and gene and SNP specific TaqMan MGB probes to simplify experimental procedures and reduce laboratory time consumption in reaching the correct diagnosis. This method has the potential to become a complementary diagnostic tool in detecting mono or bi-allelic segregation of deleterious point mutations in recessive inherited diseases.

P180

DL922-947 MODULATION OF TUMOR ASSOCIATED MACROPHAGE POLARIZATION IN TRIPLE NEGATIVE BREAST CANCER

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BACKGROUND-AIM

Triple-negative breast cancer (TNBC) is a BC subtype with a poor prognosis and an aggressive phenotype. Current treatments are insufficient to cure TNBC thus novel therapeutic approaches are needed. We have widely investigated the use of the oncolytic viruses (OV) dl922-947 known to infect and replicate specifically in cancer cells determining the lyses. In TNBC cell dl922-947 induces cell death in vitro. However, the impact of dl922-947 on TNBC microenvironment is quite unexplored.

METHODS

We have assessed the modulation of IL-6 and CCL5 by ELISA assay on cell free supernatant of TNBC cell line (MDA-MB231) treated with dl922-947 at different time points (24h, 48h, 72h). In order to evaluate cell differentiation as cell viability (MTT assay) of macrophages in presence of conditioned media (CM) derived from TNBC cells we have set up a differentiation protocol of THP-1 (human monocytic cell line):
-THP-1 cells are exposed to PMA (30ng/ml) for 3 days to obtain classical macrophages (M0) then exposed for 24 hour to CM of dl922/947 untreated/ treated TNBC cells (48h, 72h). LPS +IFNg (10ng/ml, 5 ng/ml) or IL-4 (25 ng/ml) have been used as control stimuli for M1 and M2 phenotypes respectively.

RESULTS

dl922-947 treatment reduces CCL-5 and IL-6 secretion in TNBC cell line MDA-MB231. Macrophages exposed to CM of TNBC cells does not exhibit altered plate adhesion and spreading capabilities. Changes in cell morphology observed in presence of CM are comparable to acquired morphology observed in M1 activated macrophages. MTT assay show a slight increase of cell viability in macrophages exposed to CM of dl922/947 treated BC cells (at 48h) compared to untreated cells.

CONCLUSIONS

The role of tumor microenvironment is increasingly acknowledged for TNBC. Soluble factors (CCL-5 and IL-6) secreted by BC cells contributes to a pro-tumorigenic microenvironment development and TAMs play key roles in breast tumor progression and therapeutic resistance. OVs therapy efficacy is closely related to the TAM phenotype present in TME, evaluation of mechanisms by which OVs and macrophages interact to yield effective responses, could reveal unknown mechanism responsible for acquisition of drug resistance and help to predict therapy response.

P181

TRYPTOPHAN 2,3-DIOXYGENASE CHARACTERIZATION IN HUMAN MELANOMA BIOLOGY: POSSIBLE INVOLVEMENT IN CANCER STEM CELL PHENOTYPE

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BACKGROUND-AIM

The heme-enzyme tryptophan 2,3-dioxygenase (TDO) is involved in the first and rate-limiting step of tryptophan (Trp) catabolism. Recently, TDO has been associated with cancer stemness and a malignant phenotype. TDO overexpression positively correlates with CD44 expression in bladder cancer and in renal cell carcinoma. However, there are no experimental or clinical data on TDO involvement in melanoma stemness and its association with cancer stem cell (CSC). We demonstrated that TDO is upregulated by dexamethasone (dex) in the human melanoma cell line SK-Mel-28, regulating its proliferation and migration. Moreover, dex has been demonstrated to favour the development of a stemness phenotype in ovarian cancer, strengthening the intriguing and controversial role of glucocorticoids' (GCs) role in solid tumors. Our aim is to characterize TDO expression and function in human melanoma cells with different aggressiveness. Since no studies report whether GC/dex could interfere with melanoma stem cells behaviour via TDO, we propose to investigate the relationship between dex, TDO and melanoma stemness.

METHODS

By immunofluorescence detection and FACS we assessed TDO and CSC markers (CD44, CD133, CD24, GD2 ganglioside) expression in SK-Mel-28 e A375 human melanoma cell lines. Furthermore, we evaluated the effects of TDO inhibition and GC on the CSC markers expression in SK-Mel-28 and A375 by FACS, using the selective TDO inhibitor 680C91 and dex.

RESULTS

TDO is expressed in A375 cells and dex modulates it. In both melanoma cell lines more than 90% of the population showed a remarkable expression of CD44 biomarker; in particular, SK-Mel-28 cells exhibited a strong CD44 fluorescence in basal conditions rather than A375 cells. Dex treatment does not alter CD44 expression, while the TDO selective inhibitor increased it. Ganglioside GD2 was detectable in a restricted subpopulation in both melanoma cell lines, although the percentages of GD2+ A375 cells were higher than that of SK-Mel-28. Evaluation of other markers showed that SK-Mel-28 cells were negative for CD133 and CD24 expression.

CONCLUSIONS

TDO is expressed by human melanoma cell lines with different aggressiveness, and it is involved in melanoma stem cell phenotype.

P182

ROLE OF ERG1 K+ CHANNEL IN LYMPHOCYTE DEVELOPMENT AND IN NEOPLASTIC TRANSFORMATION

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BACKGROUND-AIM

It is well established that different types of ion channels, play a relevant role both in the normal process of T- and B-cell development, selection and function[1], as well as during lymphoblast's neoplastic transformation[2].
In our laboratory we have provided evidence that ERG1b, an isoform of the ether-a-gò-gò-related gene 1, encoding for a K+ channel, is overexpressed in myeloma and leukemias, where its expression correlates with a worse prognosis both in AML and ALL[3].
The current hypothesis is that these facts can be traced back to a relevant role exerted by ERG1 at specific stages of lymphopoiesis.
In this scenario our aim is to characterize the expression of ERG1 during early stages of lymphopoiesis and study how it affects B and T lymphocytes development, and the leukemogenic process.

METHODS

We have characterized an Erg1b KO mouse model, studying developing B and T cells by cytofluorimetric techniques Erg1 expression and Ca2+ measurements have been analyzed at FACS.

RESULTS

Preliminary studies on mice carrying a selective deletion of the Erg1b gene showed an unexpected block in the lymphocyte development both in the B and T lineages respectively at the proB and DN stages.
Furthermore, BM cells of Erg1b KO mice displayed a reduced capacity to develop colonies in vitro.
In the KO transgenic model, the signaling underpinning the lymphocytes differentiative mechanisms was found affected. Particularly we noticed a reduction in the level of AKT phosphorylation in the bone marrow.
In WT animals, ERG1 was found expressed in the later stages of B (IgM+) and T (DP) cell development in BM and thymus, when the selection processes occur.

CONCLUSIONS

Our results indicate an unprecedented physiological role of ERG1 in the processes of differentiation, selection, proliferation, and migration of lymphoid progenitor cells.
Furthermore, the developmental block related to Erg1 dysregulation might represent an initial step in the leukemic transformation. Additional studies are being conducted to elucidate how ERG1 K+ channel dysregulation affects lymphocyte signaling.

P183

TESTOSTERONE BINDING TO POLYQ-EXPANDED AR CAUSES SKELETAL MUSCLE DAMAGE AND ABERRANT EXCITATION-CONTRACTION COUPLING IN SBMA

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BACKGROUND-AIM

Spinal and bulbar muscular atrophy (SBMA) is an X-linked neurodegenerative disorder caused by polyglutamine (polyQ) expansions in the androgen receptor (AR) gene causing loss of lower motor neurons and progressive muscle atrophy. AR is a transcription factor activated by testosterone. PolyQ expansion alters the native function of AR, resulting in gene expression dysregulation. Clinical and experimental evidence highlights a primary role for skeletal muscle in disease pathogenesis but the molecular processes still poorly understood.

METHODS

We recently generated and characterized a mouse model carrying a pathogenic polyQ expansion in the AR that recapitulate several aspects of SBMA. We used in vivo and ex vivo electrophysiology to assess neuromuscular transmission and intrinsic muscle force. RT-PCR to evaluate the translation and imaging for the expression and distribution of excitation-contraction coupling (ECC) machinery. Finally, we evaluated intracellular Ca2+ dynamics by live-imaging and mitochondrial function with seahorse in isolated myofibers. Orchidectomy was performed to assess the androgen dependence of these alterations. The translability to human SBMA was assessed by analyzing muscle biopsies from patients.

RESULTS

We found that the expression of polyQ-AR alters intrinsic muscle force generation, which is an early event occurring before denervation. This was associated with disrupted muscle architecture and aberrant ECC genes expression in transgenic mice and SBMA patients. These early gene expression changes were associated with altered contraction/relaxation dynamics, stimulation-induced mitochondrial calcium accumulation, and aberrant myofibers respiration. Importantly, surgical castration restored ECC gene expression back to normal and ameliorated muscle architecture and mitochondrial respiration.

CONCLUSIONS

In our model, we find the skeletal muscle as a primary site of pathological changes. Our observations reveal an unpredicted role for AR in the regulation of expression of genes involved in muscle contraction and Ca2+ dynamics, which are disrupted in SBMA muscle, yet restored by castration. Importantly, these results point out muscle as a possible site of a therapy, with the ECC machinery representing a novel target to attenuate SBMA muscle degeneration.

P184

UP-REGULATION OF CYCLOOXYGENASE-2 (COX-2) EXPRESSION BY TEMOZOLOMIDE (TMZ) IN RESISTANT HUMAN GLIOBLASTOMA (GBM) CELLS

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BACKGROUND-AIM

Development of TMZ-resistance remains a main limitation in the treatment of GBM and contributes to the dismal prognosis. TMZ is an alkylating agent whose cytotoxicity is mostly due to O6-methylguanine-induced DNA damage. O6-methylguanine-DNA methyltransferase (MGMT), a key enzyme able to directly remove alkyl groups from the O6 position of guanine, whose expression is determined by methylation status of the MGMT gene promoter, is an important factor in TMZ response. COX-2 has been implicated in GBM tumorigenesis, progression, stemness potential. COX-2 inhibitors are considered a useful GBM adjunct treatment due to their ability to increase TMZ sensitivity. According to our knowledge, there is no evidence on the ability of TMZ to influence COX-2 expression in GBM. Here, we investigated the effect of TMZ on COX-2, ß-catenin, and MGMT expression in a MGMT positive GBM cell line, T98G. U251MG line was used as negative control, being both MGMT and COX-2 negative cells. The effects of NS398, a COX-2 inhibitor, alone or combined with TMZ, were also assessed.

METHODS

GBM cells were treated with TMZ and NS398 alone or in combination. Proliferation was assessed by IncuCyte® system, cell cycle profile/apoptosis by flow cytometry, clonogenic potential by colony formation assay. COX-2/ß-catenin/MGMT expression was evaluated by real-time PCR, western blotting, flow cytometry, or immunofluorescence, and PGE2 levels by ELISA.

RESULTS

TMZ significantly increased COX-2 expression/activity in T98G cells. TMZ effect could be totally prevented by co-treatment with NS398. The results with U251MG confirmed the NS398 specificity. The analyses of cell growth rate/ cell cycle/apoptosis/clonogenic potential, confirmed that COX-2 inhibitor counteracted TMZ-resistance of T98G cells. NS398 was able to prevent MGMT- and ß-catenin-upregulation induced by TMZ.

CONCLUSIONS

Altogether, our results strongly support the role of the COX-2/PGE2 system in TMZ-resistance. Moreover, as far as we know, this is the first evidence that TMZ induces COX-2 up-modulation in TMZ-resistant GMB cells. Although further studies are needed to gain a complete picture of the actors involved in the observed effects, overall, our data help to broaden the complex interplay of TMZ-resistance.

P185

RENAL CELL CARCINOMA: SENESCENCE AND PERITUMORAL MICROENVIRONMENT

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BACKGROUND-AIM

Chronic inflammation and cellular senescence (Inflammaging) may be considered a double-edged sword mechanism affecting all stages of tumor development and growth. Senescent cells can stimulate cancer progression by enriching tumor microenvironment (TME) with senescence-associated secretory phenotype (SASP). Here, we explored the possible co-expression of pro-inflammatory and SASP-related factor in the context of clear cell Renal Cell Carcinoma (ccRCC).

METHODS

To this aim, we analyzed the expression of pro-inflammatory (PTX3) and SASP-related proteins (p16/ CIP1/WAF1, p21/INK4a, IL-6) both in normal renal proximal tubular epithelial cells (RPTECs) under hypoxia conditions and in RCC cell lines (western-blot). Moreover, we evaluated the co-localization of PTX3 with SASP-related proteins within the peritumoral area of renal samples from 10 patients undergone radical nephrectomy for RCC (confocal microscopy).

RESULTS

Although absent at basal conditions, after induction of 1% hypoxia PTX3 with SASP-related proteins resulted significantly and progressively increased in RPTEC in a time-dependent manner ($p<0.01$). On the contrary, RCC cell lines showed higher levels of PTX3 and IL-6, as compared to RPTEC at baseline ($p<0.05$ for both), while cell-cycle inhibitors were undetectable.

At tissue level, PTX3 expression was up-regulated in both RCC peritumoral and cancer tissues, as compared to normal renal tissues ($p<0.05$). The analysis of SASP revealed the co-expression of PTX3 and cell cycle inhibitors p21 and p16 in all samples, mainly at tubular level. In particular, p16 expression was higher as compared to p21 ($p<0.05$). Notably, PTX3 expression was increased in both peritumoral and cancer tissues compared to normal tissues, while p21/p16 expression was increased in peritumoral tissues compared to normal tissues but decreased in cancer tissues. In addition, p16 and IL-6 were co-expressed in the peritumoral area, while IL-6, but not p16, levels remain elevated in RCC tissues.

CONCLUSIONS

These preliminary data suggest a possible key role of inflammaging for RCC progression. Apparently normal peritumoral tissue seems to develop a senescent status in the attempt to avoid malignant transformation promoting cell cycle arrest, while SASP phenotype ends up inducing tumor growth and inflammation.

P186

ANALYSIS OF THE ANTIBODY RESPONSE AGAINST SARS-COV-2 IN VACCINATED PEOPLE AND IN RECOVERED PATIENTS

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BACKGROUND-AIM

Deciphering the antibody production against SARS-CoV-2 is essential for understanding the immune response in vaccinated subjects and in recovered patients. Here we analyze the laboratory findings of 345 patients to describe possible correlations between SARS-CoV-2 antibodies eceptor-binding domain (RBD)- as IgG and the medical history of the patients recovered .In this study we evaluated 197 vaccinated patients, with the second dose carried out between 10 and 20 days before testing, 44 people who had taken the second dose between 20 and 40 days , 36 people between 40 and 80 days and 20 people between 80 and 120 days after the last dose of the anti vaccine SARS-CoV-2.We also analyzed the antibody response in 50 people recovered from COVID19 infection at various time course and divided with respect to the duration of infection.

METHODS

The analysis was performed using the Maglumi 2000 Snibe®, a chemiluminescence analytical system with proprietary software. The performances declared by the manufacturer are: sensitivity equal to 78.65% for IgM and 91.21% for IgG; specificity equal to 97.50% for IgM and 97.3% for IgG (2) (3).

RESULTS

Out of all vaccinated patients, we had 65% of women and 35% of men, with an average age of 48 years. 97% carried out 2 doses of Pfizer vaccine. Of these, 58% had no adverse reactions after the second administration, 30% only mild adverse events and 12% moderate adverse events.
In the 1° group of vaccinated people (10-20 days after the second dose) we found an average of [Ig] RBD of 2962 BAU / mL (Binding Antibody Unit, WHO standards). In the 2° group (20-40 days) we observed a [IgG] anti RBD mean of 1511BAU / mL. In the 3° group (40-80 days) the mean concentration of anti RBD igG was 1135 BAU / mL. In the last group (80-120 days) of vaccinated people the average concentration was 310 BAU / mL.
In subjects recovered from SARS-CoV-2 infection we found an inverse correlation between the days that elapse between negativization and the IgG antibody dosage and a higher mean titer in those who had a protracted infection (greater than 35 days).

CONCLUSIONS

The progressive decrease over time of the concentration of anti sars-cov-2 IgG and the low titre of some subjects requires an analysis and monitoring of the mediated T-Cell response

P187

EFFECTS OF BLUEBERRY BIOACTIVE COMPOUNDS IN ORAL SQUAMOUS CARCINOMA CELL LINES

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BACKGROUND-AIM

Oral squamous cell carcinoma (OSCC) represents more than 90% of all oral tumors. The five-years survival rate has not improved significantly in recent years since most patients are diagnosed at advanced stage and chemotherapy regimens are limited and often ineffective. Tobacco represents an exogenous source of ROS and a link between free radical levels, oxidative stress and the pathogenesis of oral mucosa diseases has been widely demonstrated. Moreover, combined expression of EGFR, ErbB2 and ErbB3 may help predict the reduced survival of OSCC patients.
Phytochemicals are a large class of plant secondary metabolites with antiinflammatory, antioxidant and antimicrobial functions. Some of them showed anticancer efficacy as adjuvant chemotherapeutic agents. The potential health benefits of Blueberry (BL) are attributed to antioxidant properties of its bioactive compounds, in particular anthocyanins and ascorbic acid.
The present study aimed to investigate the effects of BL on cell viability and gene expression of ErbBs and molecules involved in oxidative stress in OSCC cell lines.

METHODS

The effects of BL on CAL27 (human tongue carcinoma) and A253 (human salivary gland carcinoma) cells proliferation and death were assessed by MTS assay. The impact of the treatments on cell cycle distribution and apoptosis was analyzed by flow cytometry. Gene expression was evaluated by RT-qPCR.

RESULTS

MTS assay revealed that BL treatment significantly reduced cell viability in CAL27 and A253 cells; in particular, in A253 cells, the reduction of cell viability was greater at lower concentrations of BL. FACS analysis showed that treatment with BL slightly increased the apoptotic response, especially in CAL27 cells. In A253 cells, BL treatment caused a reduction in cell migration compared to untreated cells. CAL27 cells treated with different concentrations of BL showed a reduction in ErbB2 and CAT gene expression and an increased expression of EGFR. Treatment of A253 cells with BL induced a significant increase of EGFR and ErbB2 genes, which however decreased at higher concentrations.

CONCLUSIONS

These preliminary results highlight a different modulation of ErbB genes and antioxidant molecules in response to BL and deserve to be further investigated to understand the mechanisms that regulate the observed modulations.

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