S.I.R.T.E.P.S.
SOCIETÀ ITALIANA
RICERCA TRASLAZIONALE E PROFESSIONI SANITARIE

2ND MEETING S.I.R.T.E.P.S.
SOCIETÀ ITALIANA RICERCA TRASLAZIONALE
E PROFESSIONI SANITARIE

TRANSLATIONAL RESEARCH, BIOTECHNOLOGY AND HEALTH CARE: A WINK TO THE FUTURE

L'AQUILA, 3-4 NOVEMBER 2022

AULA MAGNA " ALESSANDRO CLEMENTI "
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UNIVERSITÀ DEGLI STUDI DELL'AQUILA

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Dipartimento di Scienze Cliniche Applicate e Biotecnologiche

MESVA
Dipartimento di Medicina Clinica, Sanità Pubblica, Scienze della Vita e dell'Ambiente

CONFERENCE AIM

The 2ND Meeting of S.I.R.T.E.P.S. aims at bringing together leading academic scientists, researchers and research scholars to exchange and share their experiences and research results on all aspects of advanced medical technologies and biotechnologies applied to translational research, diagnosis and therapies.

The meeting is spread over two days and includes an opening lecture and three thematic sessions with keynote speakers and selected oral presentations. A poster session will also be present, in order to provide a more fruitful opportunity of interactions and collaborations among participants.

During the meeting closing session, the S.I.R.T.E.P.S. Board of Director will award the best oral presentation and the best poster with the S.I.R.T.E.P.S. best oral presentation prize “Alberto Gulino” and the S.I.R.T.E.P.S. best poster prize “Pierluigi Morosini”.

We hope you will enjoy the Conference and we look forward to meeting you in L’Aquila!

Francesca Zazzeroni
Lia Ginaldi
Daria Capece

PROGRAMME

THURSDAY, 3 NOVEMBER 2022

12:00-14:00 Participant Registration, Poster

14:00-14:30 Authority's Greeting

14:30-15:00 Message of Presidents Prof. Mauro Giovanni Carta and Prof. Giorgio Stassi

OPENING LECTURE

15:00-15:50 **Luigina Romani, University of Perugia**

Microbiota Medicine: Towards Clinical Revolution

SESSION I: **Organoid Technology to Model Disease**
Chairs: Cinzia Marchese, Benedetta Cinque

16:00-16:30 **Vincenzo Corbo, University of Verona**
Organoids to Model Pancreatic Cancer Evolution under Suboptimal Niche Conditions

16:30-16:45 Fabiola Marino, University Magna Graecia of Catanzaro
The Next Generation of Model Systems of Human Cardiac Organoids in a Dish [P36]

16:45-17:00 Giulia Cantini, University of Florence
Novel 3D In Vitro Models for the Study of Adrenal Diseases [P09]

17:00-17:15 Simone Di Franco, University of Palermo
Role of bidirectional crosstalk between adipose and cancer cells in obese CRC patients' progression [P19]

17:15-17:30 Maddalena Mastrogiacomo, University of Genova
Tumor-derived Extracellular Matrix Allows the Creation of a Patient-personalized In Vitro Tumor [P37]

17:30-18:15 **POSTER SESSION & COFFEE**

18:15 **Assemblea dei Soci ed Elezione del Consiglio Direttivo S.I.R.T.E.P.S.**

20:00 **Cena Sociale**

FRIDAY, 4 NOVEMBER 2022

SESSION II: **INNOVATION IN TRANSLATIONAL RESEARCH FOR REHABILITATION**
Chairs: Paolo Pillastrini, Giovanni Morone

9:00-9:30 **ANDREA TUROLLA, UNIVERSITY OF BOLOGNA**
Innovative Technologies and Modalities for Sensory-Motor Rehabilitation in Neurological Conditions

9:30-9:45 ALESSIA BRAMANTI, UNIVERSITY OF SALERNO
Biomarkers as a New Model to Assess the Efficacy of Cardiac Telerehabilitation though Artificial Intelligence [P06]

9:45-10:00 GIULIA COSSU, UNIVERSITY OF CAGLIARI
Active Elderly and Health, Bio-Psycho-Social Approach and New Rehabilitation Technologies [P13]

10:00-10:15 ALESSANDRA PERRA, UNIVERSITY OF CAGLIARI
Virtual Reality Frontiers in Bipolar Disorders: a Recovery Oriented Cognitive Rehabilitation Tool [P42]

10:15-10:30 GIUSEPPE LANZA, UNIVERSITY OF CATANIA
Non-invasive Brain Stimulation Techniques in Sleep Disorders: from Neurophysiology to Neuromodulation [P31]

10:30-11:15 **POSTER SESSION & COFFEE**

SESSION III: **ADVANCED OMICS BIOTECHNOLOGIES**
Chairs: Gianandrea Pasquinelli, Alessandra Tessitore

11:15-11:45 **GASTONE CASTELLANI, UNIVERSITY OF BOLOGNA**
Machine Learning and Artificial Intelligence Methods for Multi-Omics Integration

11:45-12:00 CLAUDIA DE VITIS, "SAPIENZA" UNIVERSITY OF ROME
ALDOC- and ENO2- driven Glucose Metabolism Sustains 3D Tumor Spheroids Growth Regardless of Nutrient Environmental Conditions: a Multi-Omics Analysis [P18]

12:00-12:15 MARÍA LABRADOR, UNIVERSITY OF TORINO
Functional Screenings Converge on MDM2 as a Modulator of Proteasome Inhibitor Resistance in Multiple Myeloma [P30]

12:15-12:30 ANNALISA ASTOLFI, UNIVERSITY OF BOLOGNA
Molecular Profiling of SDHA-deficient GIST Guides Preclinical Disease Modeling Based on Induced Pluripotent Stem Cells [P02]

12:30-12:45 LUDOVICA LOSPINOSO SEVERINI, "SAPIENZA" UNIVERSITY OF ROME
Proteomic Analysis Revealed SALL4 as a Substrate of CRL3REN Complex and a New Therapeutic Target for Sonic Hedgehog-dependent Medulloblastoma [P33]

12:45 **AWARD CEREMONY AND CLOSING REMARKS**

2ND MEETING S.I.R.T.E.P.S.
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P01.

An ex-vivo Model to Evaluate the Effect of Rare Mutations and Drug responsiveness in patients with Cystic Fibrosis

Felice Amato[1,2], Valeria Rachela Villella[1,2], Immacolata Zollo[1,2], Speranza Esposito[2], Antonella Miriam Di Lullo[3], Monica Gelzo[1,2], Filippo Scialò[1,4], Gustavo Cernera[1,2], Federica Zarrilli[1,2] and Giuseppe Castaldo[1,2]

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[4]Dept of Traslational Medicine, Università della Campania L. Vanvitelli, Naples, Italy.

To date, about 2000 genetic variants have been reported in the CFTR gene but the disease-liability of all these variants has been completed only for ~400 of the most common ones. The diagnosis of Cystic Fibrosis (CF) is supported by clinical symptoms, abnormal sweat chloride test and by the identification of two CFTR disease-causing mutations. The use of next generation sequencing (NGS) in molecular diagnostics has allowed incredible progress in the identification of CFTR genetic variants with high accuracy and a significant cost reduction. However, the interpretation of genetic variations represents one of the limiting phases of NGS technologies, also because thousands of rare variants are without pathogenic effects. In addition, the advent of new drugs, which can correct the basic defect of the CFTR protein, is changing the fate of many patients, but not all of them. Indeed, these new drugs are currently available for patients with a specific subset of mutations, and again, patients with rare or ultra-rare mutations are the most penalized. Thus, despite the great contribution of NGS in the Cystic Fibrosis diagnosis, a common effort is needed, through appropriate methodologies, to accurately and quickly define the effect of these rare mutations and their drug responsiveness. We developed a functional studies platform, based on an ex vivo analysis of human nasal epithelial cells (hNECs), to unveil both the physio-pathological effect of novel/rare mutations and the responsiveness of patients to approved drugs or putative novel ones. This functional studies platform is composed of an interdisciplinary approach that takes advantage of different expertise in the field of cell and molecular biology, biochemistry, genetics, and clinicians. The hNECs can be easily obtained from patients, including those in early pediatric age and can be either used to study the effect of mutations of uncertain significance and to measure the responsiveness of CF Patients to therapy. Here I report the data obtained from the application of this type of approach on some patients with rare mutations and how this has made possible the treatment of these patients with the current therapies, for which they had been excluded based on their genotype.

P02.

MOLECULAR PROFILING OF SDHA-DEFICIENT GIST GUIDES PRECLINICAL DISEASE MODELING BASED ON INDUCED PLURIPOTENT STEM CELLS

Astolfi Annalisa[1], Schipani Angela[2], Indio Valentina[3], Nannini Margherita[1], Urbini Milena[4], Palumbo Teresa[5], Costa Alice[1], Gozzellino Livia[1], Pasquinelli Gianandrea[1,6], Pantaleo Maria Abbondanza[1,7]

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[2]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Nederland
[3]Department of Veterinary Medical Sciences, University of Bologna, Italy
[4]Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy
[5]Alma Mater Institute on Healthy Planet, University of Bologna, Italy
[6]Division of Pathology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy
[7]Division of Oncology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract, arising from the interstitial cells of Cajal (ICCs) or their precursors. While the vast majority of GISTs harbor KIT or PDGFRA mutations, 10 –15% of GIST do not show any of these driver mutations. Among them, 20% to 40% carry mutations in one of the four SDH-complex subunits, with SDHA mutations as the most frequent event. Representative features of SDH-deficient GISTs are gastric localization, frequent lymph node metastases and an indolent clinical course despite advanced disease status. This study was aimed at uncovering the specific gene expression profile of SDHA-deficient GIST and at developing a cellular model of this disease to allow preclinical drug testing. We analyzed 36 GIST tumor samples including SDH-deficient and KIT-mutant GISTs by gene expression arrays or RNA sequencing. SDHA-deficient GISTs displayed a very homogeneous gene expression profile, different from that of KIT-mutant GIST, characterized by an increased expression of neural markers and by the activation of the hypoxia signature and of the fibroblast growth factor pathway. This specific molecular signature was used to validate a SDHA-deficient GIST cellular model obtained from induced Pluripotent Stem Cells (iPSC). Patient-derived cells were reprogrammed to pluripotency then differentiated towards the mesodermal layer, as shown by lineage marker expression (T, MIXL1, NCAM), and treated with an irreversible chemical inhibitor of succinate dehydrogenase at concentrations that inhibit mitochondrial activity (3-Nitropropionic acid, 3-NPA). Pluripotent and mesoderm-committed iPSC exposed to 3-NPA showed a statistically significant upregulation of hypoxia-related genes such as HIF1α, EPAS1 and VEGF and of neural lineage markers (LHX2, CDH2 and NEFL) whose overexpression was previously found to characterize SDHA-mutant GIST. Notably, succinate dehydrogenase inhibition in iPSC also significantly increased expression of IGF1R, a characteristic marker of SDH-deficient GISTs. Overall, this study revealed the gene expression landscape of SDHA-deficient GISTs and provided evidence that the iPSC model has the potential to mimic the molecular phenotype of the disease, therefore providing a useful tool for preclinical pharmacological testing.

P03.

HYBRID CELLS GENERATED BY BREAST CANCER CELLS ENGULFING MESENCHYMAL STEM/STROMAL CELLS ENHANCECHEMO-RESISTANCE AND METASTASIS

Giuseppina Augimeri[1,2], Maria E. Gonzalez[2,3], Alessandro Paolì[1,2], Sabra Djomehri[2,3], Yu-ChihChen[4], Euisik Yoon[3,5], Shantosh Karthikeyan[6], Sooryanarayana Varambally[6], Johanna M.Buschhaus[7], Gary D. Luker[3,7], Loredana Mauro[1], Daniela Bonofiglio[1], Sebastiano Andò[1], and Celina G. Kleer[2,3]

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[5]Department of Electrical Engineering and Computer Science and Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA.
[6]Molecular and Cellular Pathology, Department of Pathology, University of Alabama at Birmingham, Birmingham, 35233, AL, USA.
[7]Center for Molecular Imaging, Department of Radiology, University of Michigan, Ann Arbor, MI, USA.

The establishment of chemo-resistance represents the major limitation for breast cancer therapy,leading to patients' relapse and death. We previously documented the presence of Breast Cancer Cells(BCCs) that engulf Mesenchymal Stem/Stromal Cells (MSCs) in clinical samples of breast cancer metastasis and we showed that MSC engulfment leads to a more aggressive breast cancer phenotype.However, the phenotypic features of BCC-engulfing MSC are still unclear. Herein, we show that clinicalsamples of chemo-resistant breast cancer metastasis contain a hybrid cancer cell population co-expressing cytokeratin and the MSC marker Fibroblast activation protein-α (FAP). Using a co-culturemodel of patient-derived MSCs and BCCs, we demonstrate that MSC engulfment by BCCs generates ahybrid multinucleated cell population characterized by BCC/MSC features and senescence-associatedsecretory phenotype which exhibit chemo-resistance and metastatic progression in vitro and in vivo.Our data unravel that hybrid cells acquire resistance to chemotherapy and drive the metastatic growthduring drug treatment providing new insight into the mechanism of breast cancer chemo-resistance.

P04.

DEVELOPMENT OF A BONE-ON-A-CHIP BASED ON A 3D OSTEOCYTIC NETWORK FOR THE SCREENING OF ANTI-OSTEOPOROTIC DRUGS

S. Avnet[1,2], M.V. Lipreri[1], G. Di Pompo[2], G. Graziani[2], E. Boanini[3], N. Baldini[1,2]

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Introduction and Objective. The osteocyte, recognized as a major orchestrator of osteoblast and osteoclast activity, is the most important key player during bone remodeling processes. Imbalances that occur during bone remodeling, caused by hormone perturbations or alterations in mechanical loading, can induce bone disease as osteoporosis. Due to limited understanding of the underlying mechanisms, current therapies for osteoporosis cannot adequately address this imbalance because current studies of osteocytes rely on conventional cell culture that cannot recapitulate local in vivo microenvironments for the lack of control of the spatial/temporal distribution of cells and biomolecules. Microfluidics is the science and technology of microscale fluid manipulating and sensing and can help fill this gap.
Materials and Methods. We used a microfluidic device to enable the culture of osteocyte-like cells(MLO-Y4 and MLO-A5) in a 3D fashion. Osteocytes were cultured in a perfused and 160 µm high channel and embedded in a bone-like extracellular matrix: osteocytes were embedded in a matrigel-and collagen-based hydrogel enriched with nanostructured hydroxypatite crystals (HA-NP) to mimic bone. To set up the best combination of matrigel enriched with Type I collagen we used fluorescent microspheres and confocal analysis. To evaluate the viability and the expression of osteocytic markers, we used live-dead assay and immunofluorescent staining and confocal analysis combined with automated quantification. For mineralization, we performed alizarin red staining. Results. Osteocytes in the organ-on-a-chip model showed high viability and, in respect to 2Dconventional cell cultures an increased differentiation, as assessed by a live-dead assay and the staining of the osteocytic markers connexin-43 and alkaline phosphatase and the increased mineralization activity. Furthermore, the addition of HA-NP significantly increased the formation of dendrite-like structures spreading through the xyz-axes, as assessed after G-actin immunofluorescence.
Conclusions. Using a microfluidic device for MLO-Y4 and MLO-A5 cell cultures, compared to the 2D surfaces, we demonstrated a significant difference in cell differentiation and morphology. In particular, 3D cultures allowed the formation of 3D cell networks and the osteogenic phenotype. As a platform technology, this microfluidic device can function as a novel cell culture model that enables further studies of osteocytes and 3D co-culturing with other bone cells for the screening of anti-osteoporotic drugs.

P05.

INNOVATIVE TECHNIQUES FOR MONITORING AND ENHANCING REHABILITATIVE EFFECTS: A STUDY IN PEOPLE WITH MULTIPLE SCLEROSIS

Monica Biggio[1], Ludovico Pedullà[1,2], Andrea Tacchino[2], Costanza Iester[3], Giampaolo Brichetto[2], Ambra Bisio[1], Marco Bove[1], Laura Bonzano[3]

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Multiple Sclerosis (MS) is a chronic disease of the central nervous system characterized by inflammation, demyelination, axon loss and gliosis. Some patients show a rapid exacerbation of mobility disorders and gait impairment severely affecting the quality of life with the need to use a walking aid. To date, rehabilitation is the only possible approach, with particular regard to lower limbs mobility, gait function and the proper use of the walking aid. Here, we aim to compare the behavioral and neural effects of a standard rehabilitation protocol in a group of people with MS (PwMS) with walking impairment through the use of innovative techniques. In particular, the evaluation process includes a neuroimaging technique, i.e., functional Near-Infrared Spectroscopy (fNIRS), to investigate cortical activity during different walking tasks.
From a behavioral point of view, during the rehabilitation period PwMS use a prosthetic aid (a sensorized cane called FBKEIN) able to monitor for a prolonged period of time (i.e. months) the user's gait and to administer biofeedback stimulation helping the user to properly use the walking aid. A longitudinal study of 30 months was designed. The inclusion criterium was Expanded Disability Status Scale - EDSS≥6 (i.e., use of walking aids). The study design consists in alternating rehabilitation (Rehab) and waiting list (NoRehab) periods. The rehabilitative intervention includes exercises for lower limb mobility and postural stability, motor control tasks and gait rehabilitation. Patients are randomized into 2 homogeneous groups, both using FBKEIN for the whole period of participation in the study in order to record dynamic and kinematic parameters during gait. However, only the experimental group receives biofeedback stimulation in the NoRehab phase. In particular, FBKEIN was developed for postural stability assessment in dynamic condition and functional recovery in gait rehabilitation. Therapists can set in FBKEIN to provide acoustic and tactile biofeedback based on load thresholds tailored for each patient. Only in the experimental group, an output signal (ringing and vibration at the hand level) is provided if the aid is used incorrectly (e.g., excessive load applied on the cane and consequent lateral displacement of weight), as a feedback during everyday life activities. Participants are assessed before and after each study phase in terms of motor function, using standard lower limb clinical tests, such as Timed 25-Foot Walk and 6 Minute Walking Test. In addition, during the evaluation phases, cortical activity during walking tasks is recorded through fNIRS, with an array composed of 50 standard channels covering prefrontal, sensorimotor and parietal areas. Twelve PwMS (age = 57.7±13.6 years) were recruited until now. They underwent the baseline assessment and started the rehabilitative intervention. Moreover, they were trained to the correct use of the walking aid. Preliminary results were obtained from the first evaluation phase. Higher cortical activation was found during curvilinear with respect to linear walking, mostly in prefrontal areas. Both PwMS and therapists reported high usability of the system. FBKEIN could be a usable tool allowing to self-monitor everyday life walking ability and providing feedback that could help maintain the effect of a rehabilitation treatment.

P06.

BIOMARKERS AS A NEW MODEL TO ASSESS THE EFFICACY OF CARDIAC TELEREHABILITATION THOUGH ARTIFICIAL INTELLIGENCE

Mariaconsiglia Calabrese[4], Rosella Ciurleo[3], Michele Ciccarelli, Albino Carrizzo[1,2], Alessia Bramanti[1]

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[2]IRCCS Neuromed, Pozzilli (IS).
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Evidence from experimental models has shown that the physical exercise is able to increase levels of Sirtuin 1 (SIRT1), an important deacetylase involved in cellular and metabolic adaptation to physical activity, counteracting the process of cellular aging. In addition, the activation of SIRT1 is able to evoke the release of Nitric Oxide (NO), the main determination of cardio-cerebrovascular well-being. Based on these results and the new concept of "Translational Rehabilitatiomic", we investigated the activity modulation of SIRT1, catalase and superoxide dismutase (SOD), enzymes responsable for neutralizing oxygen free radicals, in a court of post-infarction patients undergoing cardiac rehabilitation program (CRP). We enrolled a court of 50 elderly male patients (average age 68.6 ± 6.3 years) and divided them into two groups, non-rehabilitated (P) and rehabilitated (RP) by 4-week CRP. The levels of SIRT1, catalase and superoxide dismutase (SOD) were found to be increased exclusively in patients undergoing cardiac rehabilitation. Therefore, they could be candidates as markers able to monitor the effectiveness of physical exercise. In addition, the serum from rehabilitated patients was able to increase SIRT1, catalase and nitric oxide levels in human endothelial cells in vitro, strengthen the beneficial effect of the rehabilitation. The spread of telemedicine poses new prospects for reducing the period of hospitalization and, therefore, of national health spending, especially with the advent of telerehabilitation. So far there are no data on SIRT1, catalase and SOD levels in patients at home undergoing telecardio rehabilitation. We will define three different model of CRT telerehabilitation, in different groups of patients. Vital parameters will be recorded; blood samples will be collected at different times to assess the expression of the molecular parameters. Physical status will be analyzed. All parameters will be gathered in a data collection cloud-based for further analyses. Artificial intelligence (AI) will be exploited to integrate physical and molecular parameters aimed at define the best model of telecardio rehabilitation program to achieve the established target.

P07.

MEX3A/RIG-I AXIS AS A THERAPEUTIC TARGET FOR NEW APPROACHES IN THE TREATMENT OF GLIOBLASTOMA

Francesca Bufalieri[1], Antonino Cucinotta[1], Irene Basili[1], Miriam Caimano[1], Ludovica Lospinoso Severini[1], Francesco Paglia[2], Luigi Simpirisi[2], Daniele Armocida[3], Antonio Santoro[2], Luca D'Angelo[2],Paola Infante[1], Lucia Di Marcotullio[1]

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Glioblastoma (GB) is the most malignant primary brain tumor in human, with an overall survivalestimated from 8 to 15 months. The extensive molecular heterogeneity, as well as its rapid progression,invasiveness and the occurrence of drug-resistant cancer stem cells, limits the efficacy of the currenttherapies, making GB one of the most difficult tumor to treat. For these reasons, the identification andcharacterization of the molecular mechanisms involved in GB tumorigenesis represent a dramaticchallenge for the development of more effective and innovative therapeutic approaches.In this study, we performed a gene expression analysis on publicly available datasets to identify newmolecular players responsible for the GB malignant phenotype. We found that Muscle Excess 3A(MEX3A), an RNA-binding protein and E3 ubiquitin ligase, is strongly up-regulated in GB specimens.High levels of MEX3A are associated with very low protein levels of the Retinoic acid-inducible gene I(RIG-I), a tumor suppressor involved in the activation of the innate immune system through the type Iinterferon (IFN-I) response, and in the induction of cell growth arrest via apoptosis. We demonstratedthat MEX3A binds RIG-I and promotes its ubiquitylation and proteasome-dependent degradation.Further, the genetic depletion of MEX3A leads to an increase of RIG-I protein levels and results in thesuppression of GB cell growth both in vitro and in vivo. Interestingly, we observed that high levels ofMEX3A correlates with worse overall survival and a poor prognosis. Consistently, we found a negativecorrelation between the expression of MEX3A and the isocitrate dehydrogenase (IDH1) status and apositive correlation between MEX3A and epidermal growth factor receptor (EGFR) expression, two ofthe most important prognostic biomarkers of GB, associated with good and poor prognosis,respectively.Our findings uncover MEX3A as a novel prognostic biomarker in GB and highlight a previouslyunknown role of MEX3A/RIG-I axis in GB tumorigenesis suggesting that targeting these moleculescould open innovative perspectives for new multi-targeting approaches in the treatment of this devastating tumor.

P08.

HOME ROBOTIC REHABILITATION FOR UPPER LIMB IN PATIENTS WITH CHRONIC STROKE. A PILOT STUDY

Campagnola B[1], Bressi F[1], Cricenti L[1], Santacaterina F[1], Fiori F[1], D'Alonzo M[1], Ricci L[1], Capone F[1], Pacilli A[2], Miccinilli S[1], Di Pino G[1], Di Lazzaro V[1], Sterzi S[1], Bravi M[1]

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Background: Evidence in the literature underline how the motor system is plastic after stroke and motor training can positively influence the recovery. Robotic therapy (RT) can be a valuable aid in this, allowing to propose sessions of controlled and identical exercises, customizing settings and characteristics on the individual patient. However, the logistical and economic restrictions limit its use in hospital environments, excluding patients no longer hospitalized or living in isolated places, for which it is difficult to reach the rehabilitation site. A recent review reports that in motor recovery after stroke, telerehabilitation appears to have similar results to clinical rehabilitation. According to this review, both for sub-acute and chronic patients, technological rehabilitation programmes should be a complement to conventional therapy .
The purpose of this study is to evaluate the effectiveness and feasibility of a remotely monitored home treatment in chronic stroke patients using iCONE device (Heaxel srl, Milan, Italy).
Materials and Methods: Patients carried out an initial (T0) and final (T1) assessment with the robotic device and clinical scales. After initial evaluation, the robot was delivered to the patient's home. The rehabilitation protocol provided 10 days of upper limb at-home treatment (5 days a week for 2 weeks) under the direct supervision of a caregiver previously trained to use the device.
Results: Comparison between T0 and T1 evaluations revealed some significant improvements in robot-evaluated indices ($p<0.05$) and in elbow spasticity ($p=0.017$). No differences were found in other functional clinical scales. A general appreciation of the robot emerged: patients spontaneously asked for the addition of further sessions and to continue therapy and caregivers reported their burden of care did not increase.
Conclusion: From the data obtained, this rehabilitation seems to be promising for this population. It would be interesting to conduct RCT studies to compare a conventional treatment in structure with a robotic telematics treatment.

P09.

NOVEL 3D IN VITRO MODELS FOR THE STUDY OF ADRENAL DISEASES

Giulia Cantini[1-2], Serena Martinelli[1-2], Daniele Guasti[3], Mario Maggi[1-2], Massimo Mannelli[1], Elena Rapizzi[2-3], and Michaela Luconi[1-2]

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[2]Centro di Ricerca e Innovazione delle Patologie Surrenaliche.
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The adrenal gland is a complex endocrine organ integrating the steroidogenic cortex and the neuroendocrine chromaffin medulla. The tuned interaction between the two components regulates the development, maintenance and functional activity of the entire gland in pathophysiological conditions. The currently available in vitro and in vivo cell and xenograft models of adrenal cancers have limits, as they fail to mimic the 3D structure and complexity of the organ.

Novel models addressing the cell interactions between steroidogenic and chromaffin cells, their architecture as well as the role of the microenvironment are needed, to take a significant step forward in adrenal cancer research and developing more efficacious and targeted therapies.

Starting from human foetal adrenal samples at different gestational ages, we developed a unique in vitro human foetal cell modeling representative of all the components of the adrenal gland to study the development of this organ in both physiological and pathological conditions.

Furthermore, to study the cortical-medulla interplay in the adult tissue, we developed an in-vitro 3D functional model called adrenoid (ADR) of the whole adrenal gland, overlaying the limitations of adrenal single population approaches, to study the adrenal-associated diseases, such as adrenal tumors, and to support new drug development.

These innovative in vitro models reflect the spatial complexity and cell functionality of the adrenal gland since both components are maintained both in the foetal and in adult models. Our findings may be relevant for extending the repertoire of preclinical models for the study of endocrine tumors and useful for the development and testing of novel drugs.

P10.

THERAPEUTIC POTENTIAL OF EXOSOMES DERIVED FROM HUMAN AMNIOTIC FLUID STEM CELLS

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Stem cells offer the basis for the promotion of robust new therapeutic approaches for a variety of human disorders. There are still many limitations to be overcome before clinical therapeutic application, including a better understanding of the mechanism by which stem cell therapies may lead to enhanced recovery. In vitro investigations are necessary to dissect the mechanisms involved and to support the potential development in stem cell-based therapies. In spite of growing interest in human amniotic fluid stem cells, not much is known about the characteristics of their secretome and regarding the potential neuroprotective mechanism in different pathologies. To get more insight on amniotic fluid cells therapeutic potential, in this study the conditioned medium derived from Human amniotic fluid stem cells (CM-hAFSCs) has been characterized. In a first set of experiments, we characterized the exosomes derived from hAFSCs using Nanosight and Exoview analyses. Then, miRNAs expression profile in the exosomal fraction of the conditioned medium was examined. Specifically, microRNA analysis in the exosomal component revealed a panel of 16 overexpressed miRNAs involved in the regulation of coherent signaling pathways, including neurotrophin signaling, and those related to neuroprotection and neuronal cell death. To deep in knowledge of the neurotrophic component, we analyzed the expression of the mature form of BDNF and, interestingly, mBDNF was higher in the exosomal component. This work highlights the relevance of the use of exosomes derived from hAFSCs as therapeutic approach for neurological disorders, including stroke, Parkinson's and Alzheimer's disease.

P11.

CIRCULATING MICRORNAS AS PREDICTIVE BIOMARKERS IN FAMILIAL-HEREDITARY BREAST CANCER

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Introduction: Circulating microRNAs (miRNAs) are considered potential diagnostic and predictive biomarkers in cancer. Breast cancer (BC) is the most common cancer in women and approximately 5–10% of BC cases are classified as familial or hereditary, 25% of which are associated with germline mutations in the high penetrance susceptibility genes BRCA1 and BRCA2. Here, we performed a circulating miRNA profiling in hereditary-familial BC cases in order to identify differentially expressed miRNAs with putative value of non-invasive biomarkers and to deepen the molecular mechanisms involved in the pathogenesis of familial-hereditary BCs.
Material and Method: Plasma miRNAs of a series of 21 familial BC cases, including 14 BRCA1/2 positive (BRCA) and 7 BRCA1/2 negative (non-BRCA) BCs, were analyzed by miRNA-sequencing using Illumina technology. Three age-matched healthy controls were also included in the study.
A bioinformatic pipeline comprising Bowtie1 tool for alignment to miRBase v.22 and the reference genome GRCh38, as well as DESeq2 package for differential expression analysis was used. In silico analysis of the target/pathway genes of differentially expressed miRNAs was performed using the on-line DIANA and miRNet tools.
Results: miR-320e and miR-486-3p emerged as the most relevant miRNAs able to discriminate BC cases and controls, showing down-regulation in BCs compared to healthy controls. Consistent with these data, tumor suppressor function was ascribed to these two miRNAs, as reported in several studies. Different expression levels of mir-486-3p were also observed related to BC molecular subtypes. A panel of 20 differentially expressed miRNAs between BRCA and non-BRCA cases was also identified, of particular relevance for the non-BRCA group. In silico analysis of target/pathway genes of the differentially expressed miRNAs revealed an enrichment of genes involved in different cancer types (e.g. prostate, lung, melanoma) and key biological processes, including cell cycle, apoptosis, focal adhesion and growth factor signaling pathways.
Conclusion: These preliminary results suggest that the analysis of the expression levels of circulating miRNAs in familial-hereditary BCs, based on the BRCA1/2 germline mutational status, could provide, once extended and confirmed on a larger number of patients, important information of biological and clinical relevance, potentially exploitable in the context of screening and prevention programs.

P12.

OSTEOSARCOMA TUMOROIDS AS A MODEL TO STUDY EXTRACELLULAR MATRIX AND ITS ROLE IN CANCER AGGRESSIVENESS AD RESPONSE TO THERAPY

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The extracellular matrix is primarily composed of collagen, fibronectin, laminin, and proteoglycan andmodulates cell behavior, shapes and maintain tissue vitality, and confers mechanical properties. In thepathological state of cancer, however, ECM cultivates tumorigenesis and metastasis and provides afundamental feature of the tumor microenvironment. Its dysregulation and aberrant remodeling incancer microenvironment has gained considerable attention for its promise in pathogenic targeting andpredictive value.Here, we aim at the generation of a complex 3D organoid model of osteosarcoma, a cancer ofmesenchymal origin characterized by high levels of metastasis and relapses. To recapitulate theinteractions between cancer cells and ECM, we made use of spheroids of metastatic or non-metastaticosteosarcoma cells, mixed with cells of the tumor microenvironment, the mesenchymal stromal cells(MSC), that, as previously demonstrated, can be reprogrammed to provide tumor support. We aimed atinvestigating the role of ECM in cancer aggressiveness and in response to therapies in osteosarcoma.Mixed spheroids were grown up to 14 days to allow deposition of the ECM. We characterized thefeatures of the ECM and assessed collagen I deposition by immunofluorescence and with live stainingwith confocal dual-photon microscopy. We could demonstrate that collagen I deposition occurs at theinterphase between tumor cells and mesenchymal stromal, suggesting that cancer cells mightmodulate the secretion of ECM proteins in MSC, via IL-6. Moreover, collagen I expression was founddecreased in organoids of metastatic cells, indicating higher invasive abilities, whereas the presence ofthick ECM leads to compact organoids and reduced motility in non-metastatic cells.Our results lay the foundation for the development of osteosarcoma organoids by using patient-derived-cells for precision medicine approach, to allow future prediction of intra-patient response.

P13.

ACTIVE ELDERLY AND HEALTH, BIO-PSYCHO-SOCIAL APPROACH AND NEW REHABILITATION TECHNOLOGIES

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Europeans are living longer than ever before, and the age profile of society is rapidly developing.
In 2050 30% of the European population will be over 65 years or over. The frequency of chronic diseases will also increase over time specially in late life.
WHO guidelines indicate the need to develop therapeutic and rehabilitation strategies that promote, maintain and rehabilitate the bio-psycho-social health of older adults.
The Covid 19 pandemic has also revealed how supportive technology can be in restrictive conditions, but also how generational digital divide can expose older adults to further marginalization. The aim of the presentation is to show the results of rehabilitation and health promotion projects promoted by the University of Cagliari using:
-Telemedicine (monitoring and intervention)
-Digital communication technologies (monitoring and intervention)
-Virtual reality rehabilitation technologies (clinical protocols)
Health outcomes were detected through validated tools that investigate:
Quality of life
Depressive symptoms
Cognitive functions
Social rhythms and biorhythms
Fundings presented showed statistically significant results on old adult health outcomes following the actions implemented, facilitating relevant social inclusion processes through the use of technologies.
Enhancing actual bio-psycho-social and interdisciplinary approach in a Translational Research we have developed targeted and effective actions improving, supporting and monitoring old adult health.

P14.

EFFECTS OF THE USE OF EXOSKELETAL SYSTEMS ASSOCIATED WITH NIBS ON GAIT KINEMATIC AND SPATIO-TEMPORAL PARAMETERS IN PATIENTS WITH CHRONIC STROKE

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Gait retraining is a fundamental component of the rehabilitation. Different types of gait rehabilitation approach are described, such as overground training, treadmill training with or without load suspension and robotic assisted gait training. To enhance the effects of these training modalities, an important role is played by non-invasive brain stimulation techniques (NIBS). The present study aimed to assess whether the use of an exoskeletal robotic system in association with NIBS in chronic stroke patients enhances gait kinematic and spatiotemporal parameters. The study involved 16 patients, randomized into two groups: Real and Sham, depending on whether or not NIBS was administered prior to gait training. The treatment included 10 training sessions (for 2 weeks; 5 days/week). The spatial-temporal, kinematic, and dynamic parameters recorded through the BTS stereo-photogrammetric system were used as primary outcome measures, and clinical scale (Fugl-Meyer, Ashworth; Motor Power, FAC), test for motor function (10MWT and TUG test) and quality of life (SF-36, BDI) were proposed as secondary outcomes. Patients were assessed at 3 time point: T0 (baseline), T1 (at the end of the treatment) and T2 (at 3 months follow-up).
Preliminary data showed no significant differences at each time point, except for one patient who reported promising results, but which had different age and function characteristics compared to the rest of the enrolled population. The results therefore seem to indicate the ineffectiveness of these treatment modalities (type of instrumentation, combination with NIBS and timing of the treatment), however it is necessary to increase the sample size and the number of training sessions to verify their effectiveness.

P15.

A High Throughput Drug Screening revealed new synthetic lethal interactions to ProteasomeInhibitors in Multiple Myeloma

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Multiple myeloma (MM) is a terminally differentiated B-cell malignancy characterized by immunoglobulins overproduction. The introduction of proteasome inhibitors (PIs) as the backbone of therapeutic combinations has significantly improved patients' prognosis. Despite these enormous advances, relapses and disease progressions are common, suggesting a prominent role for either innate or acquired drug-resistance. To identify compounds that can increase the efficacy of PI in the combinatorial setting, we performed a functional screening using a library of 320 small-molecule inhibitors covering 123 key targets implicated in a wide variety of signaling pathways. The screening was performed in the PI resistant (PIR) U266PIR cell line. Cells were exposed to drugs at 4concentrations in the presence or absence of a sublethal concentration of the PI carfilzomib (CFZ) and analyzed after 72 hours for growth rate inhibition (GR) using a metabolic-based assay. Top candidates were further validated in a secondary screening carried out in 5 MM cell lines. The best synthetic lethal interactions included numerous drugs targeting pathways already known to synergize with PIs, thus confirming the efficacy of the screening. In addition, the BMP1 inhibitor UK383367 and the ATP5F1Cinhibitor Bedaquiline (BDQ) ranked among the top ten in at least 4 MM cell lines and, therefore, were chosen for further analyses. UK383367 and BDQ displayed a striking synergistic effect with CFZ in13/13 and 11/12 MM cell lines (including 4 PIR models), respectively. To exclude general toxicities, we treated peripheral blood mononuclear cells (PMBCs) extracted from 9 healthy donors. We demonstrated that combinatorial treatments with increasing doses of UK383367 or BDQ with CFZ did not affect the viability of PBMCs compared to MM cells. Next, we co-cultured the MM cell line AMO-1on a layer of HS-5 bone marrow stromal cells. We observed that CFZ combination with eitherUK383367 or BDQ enhanced MM cell death also in the presence of BM milieu, with no toxicity on HS-5cells. Overall, these preliminary data encourage further investigation on the molecular mechanisms responsible of the synergistic effect of the UK383367-CFZ and BDQ-CFZ combinatorial treatments.

P16.

COUPLING OF RHODOPSIN MUTATIONS WITH GAI IN CSNB: A NOVEL METHOD

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Autosomal dominant congenital stationary night blindness (adCSNB) is a non-progressive retinal disorder characterized by blindness in dim-light conditions and among the causes of the disease are mutations in the G protein coupled receptor rhodopsin (RHO). Photons are absorbed in rod photoreceptor by RHO, which is the visual pigment crucial for the phototransduction cascade. Mutations in RHO cause a defect in signal transmission, consequentially affecting dim-light vision without a progressive retinal degeneration.
Physiologically, 11-cis-retinal isomerize into all-trans-retinal leading to conformational changes in RHO, that activates the alpha subunit Transducin (Gt) of the heterotrimeric G protein and these are the first steps of phototransduction.
Previously, biochemically characterized RHO mutations associated with CSNB were demonstrated to cause constitutive activity of the mutant receptor with desensitization of rods in low-light conditions and constant activation of transduction. Gt belongs to the sub-family of inhibitory G protein (Gi), and crystallographic studies showed RHO-Gt but also RHO-Gi coupling.
Here we investigate the physical mechanisms that interplay in the defect of phototransduction in CSNB of two novel RHO mutations (Mut1 and Mut2) and compared to the best characterized RHO mutations for this condition, G90D and T94I. We developed a new approach to evaluate mutated RHO and Gi coupling based on Bioluminescence Resonance Energy Transfer (BRET) in COS7 cells transiently transfected with each mutant RHO and mGαi encoding plasmids. Our experiments highlighted the kinetics during time of the coupling between RHO mutants and mGαi, before and after the treatment with 10 µM 9-cis-retinal, to mimic RHO inactive state and subsequent activation, or Dimethyl-sulfoxide (DMSO) as control. BRET allowed to evaluate the different responses of RHO mutants to retinal during time and to compare responses of mutant RHO with wild type RHO (WT). While activated WT RHO binds mGαi only under 9-cis-retinal treatment (Mann-Whitney test; $p<0.05$), both Mut1 and Mut2 showed the same kinetics in presence of 9-cis-retinal or DMSO, suggesting a constitutive active state of the receptor, thus leading to its desensitization. In summary, BRET turned out to be a good system, where other tests had failed, to highlight differences between WT and RHO mutants behavior.

P17.

PHOTODYNAMIC EFFECTS OF A NEW 5-ALA GEL AND 630NM-LED ON ORAL OSTEOBLASTS AND GINGIVAL FIBROBLASTS

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Aim: The strong antimicrobial effects of new gel 5% delta-aminolevulinic acid (ALAD) associated with 630nm-LED as photodynamic therapy (ALAD-PDT) in treating periodontitis and peri- implantitis drove us to explore its potential regenerative effect on bone and soft tissues by testing this new ALAD-PDT on primary human oral osteoblasts (hOBs) and human gingival fibroblasts (hGFs).
Methods: hOBs and hGFs were cultured and incubated with ALAD gel for 45 minutes and subsequently irradiated with a 630nm-LED for 7 minutes (ALAD-PDT). To determine the time-dependent effects of ALAD-PDT treatment, the following points were evaluated: (1) cytotoxicity at 24 h (2) proliferation by MTT assay, after 48 and 72 h; (3) cellular accumulation of the photosynthesize PpIX by 0.5M $HClO_4$, after 0, 48 and 72 h; (4) ROS and the superoxide dismutase (SOD) activity at different times (5) Alkaline Phosphatase (ALP) activity after 3 days for hOBs; (6) calcium deposition by Alizarin Red Staining, and by Cetylpyridinium Chloride, after 14gg for hOBs. Cells exposed to LED as positive control. Untreated and unexposed cells as negative control.
Results: Viable and metabolic active cells were revealed at any concentrations of ALAD-PDT, but only 100-ALAD-PDT significantly enhanced the proliferation rate. A significant accumulation of PpIX immediately after 45 min of incubation (0h) was showed, but this did not increase at 48h and 72h. Higher ROS generation was detected at 10 min in hGF, and at 30 min in hOBs. The activity of the SOD enzyme augmented at 30 min in both cell types. Furthermore, the ALP activity resulted stimulated and there was a significant increase in calcium deposition of 72.33%.
Conclusions: Although further investigations are needed, the results of this study should be useful in basic research and in clinical applications. Indeed, this preliminary study shed light on the beneficial effects of ALAD-PDT on cell populations of the oral cavity. Our data highlighted the effects of 5- aminolevulinic acid on human oral osteoblasts and gingival fibroblasts. Finally, the strength of our protocol was the shortness of incubation an irradiation times compared to photodynamic therapy based on 5-ala generally proposed in the literature.

P18.

ALDOC- AND ENO2- DRIVEN GLUCOSE METABOLISM SUSTAINS 3D TUMOR SPHEROIDS GROWTH REGARDLESS OF NUTRIENT ENVIRONMENTAL CONDITIONS: A MULTI-OMICS ANALYSIS

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Background: Metastases are the major cause of cancer-related morbidity and mortality. By the time cancer cells detach from their primary site to eventually spread to distant sites, they need to acquire the ability to survive in non-adherent conditions and to proliferate within a new microenvironment in spite of stressing conditions that may severely constrain the metastatic process. In this study, we gained insight into the molecular mechanisms allowing cancer cells to survive and proliferate in an anchorage-independent manner, regardless of both tumor-intrinsic variables and nutrient culture conditions. **Methods:** 3D spheroids derived from lung adenocarcinoma (LUAD) and breast cancer cells were cultured in either nutrient-rich or –restricted culture conditions. A multi-omics approach, including transcriptomics, proteomics, and metabolomics, was used to explore the molecular changes underlying the transition from 2D to 3D cultures. Small interfering RNA-mediated loss of function assays were used to validate the role of the identified differentially expressed genes and proteins in H460 and HCC827 LUAD as well as in MCF7 and T47D breast cancer cell lines. **Results:** We found that the transition from 2D to 3D cultures of H460 and MCF7 cells is associated with significant changes in the expression of genes and proteins involved in metabolic reprogramming. We observed that 3D tumor spheroid growth implies the overexpression of ALDOC and ENO2 glycolytic enzymes concomitant with the enhanced consumption of glucose and fructose and the enhanced production of lactate. Transfection with siRNA against both ALDOC and ENO2 determined a significant reduction in lactate production and cell viability. Furthermore, both the number and size of spheroids produced by H460, HCC827, MCF7, and T47D cell lines were significantly reduced upon ALDOC and ENO2 knockdown. **Conclusions:** Our results show that anchorage-independent survival and growth of cancer cells are supported by changes in genes and proteins that drive glucose metabolism towards an enhanced lactate production. Notably, this finding is valid regardless of the tumor type and nutrient environmental availability, thus suggesting the possible general involvement of this mechanism in cancer metastasis. The pan-cancer validation of this vulnerability could potentially help to slow or prevent cancer progression.

P19.

ROLE OF BIDIRECTIONAL CROSSTALK BETWEEN ADIPOSE AND CANCER CELLS IN OBESE CRC PATIENTS' PROGRESSION.

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Obesity is a strong risk factor for cancer progression, posing obesity-related cancer as one of the leading causes of death. Nevertheless, the molecular mechanisms that endow cancer cells with metastatic properties in patients affected by obesity remain unexplored. Here, we show that IL-6 and HGF, secreted by tumor neighboring visceral adipose stromal cells (V-ASCs), expand the metastatic colorectal (CR) cancer cell compartment (CD44v6+), which in turn secretes neurotrophins such as NGF and NT-3, and recruits adipose stem cells within the tumor mass. Visceral adipose-derived factors promote vasculogenesis and the onset of metastatic dissemination by activation of STAT3, which inhibits miR-200a and enhances ZEB2 expression, effectively reprogramming CRC cells into a highly metastatic phenotype.
Together, our data suggest that targeting adipose factors in colorectal cancer patients with obesity may represent a therapeutic strategy for preventing metastatic disease.

P20.

PATHOLOGY-CHROMATIN IMMUNOPRECIPITATION (PAT-CHIP): EXPLORING THE HISTONE CODE OF HUMAN DISEASES

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Epigenomic aberrations can contribute to a pathologic state by conditioning both the gene expression and the genomic stability of the cells. The awareness of such alterations could improve our understanding of pathology dynamics and the identification of new therapeutic strategies and biomarkers to refine pathology classification and treatment. In the last decades, Formalin fixation and paraffin embedding (FFPE), the gold standard to preserve both tissue integrity and organization, allowed the accumulation of a huge number of biological samples just waiting to be molecularly dissected. Recently, we introduced new chromatin immunoprecipitation (ChIP) techniques that allow the analysis of histone post-translational modifications (PTMs) and transcription factors (TFs) distribution in FFPE tissues. The application of ChIP to genome-wide chromatin studies using real archival samples represents an unprecedented opportunity to conduct retrospective clinical studies thanks to the possibility of accessing large cohorts of samples and their associated diagnostic records. Pathology tissue chromatin immunoprecipitation (PAT-ChIP) is based on a chromatin isolation step achieved by both physical extraction, using a canonical probe sonication procedure coupled with a highly controlled micrococcal nuclease (MNase) digestion. PAT-ChIP demonstrated to work in both genome-wide (coupled with NGS) and locus-specific (i.e., real-time qPCR) studies with different tumor tissues such as seminoma, breast cancer, and lung carcinomas. The possible low efficient chromatin isolation, often due to extensive tissue fixation introduced during routine pathological processing or to old FFPE samples, may limit the PAT-ChIP application and has been overcome by introducing a heat-mediated limited reversal of crosslinking to partially revert the effects of extensive formalin fixation. This new technique was named enhanced PAT-ChIP (EPAT-ChIP) and was found to improve the success of genome-wide studies in tumor archival samples (invasive breast carcinoma). The technique has been applied with success to the study of several mouse tissues (e.g. cortex, liver, spleen, gastrocnemius) and, most importantly, to extremely critical samples such as post-mortem brain samples from two different cohorts of Alzheimer disease patients. We believe that these techniques will contribute to extend the current understanding of pathologic epigenomes, enabling the identification of pathology molecular dynamics and the development of new therapeutic strategies and clinically useful biomarkers.

P21.

CARBOXYLESTERASE 1 (CES1) IS A PROMISING DRUGGABLE TARGET IN OVARIAN CANCER

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Tumour cells show an incredible metabolic plasticity. This flexibility is a key factor that allows cancer cells to cope with the low nutrient concentrations in the tumour microenvironment, thus sustaining their proliferation and metastatic spread. Recently, carboxylesterase 1 (CES1), has been identified in colorectal carcinoma (CRC) as an essential NF-κB-regulated lipase promoting cancer-cell survival and metabolic adaptation under condition of energy stress. Specifically, CES1 was able to promote the survival of CRC cells by increasing TAG breakdown to fuel fatty acid oxidation and preventing their toxic build-up. Like CRC, ovarian carcinoma (OC) preferentially metastasizes to peritoneal cavity and infiltrates the omentum, a fat rich organ, and recent evidence pointed out the involvement of lipid metabolism in energy stress response. Therefore, we investigated whether CES1 could play an important role also in the metabolic adaptation of OC cells. Public datasets of OC patients were analysed. A panel of four OC cell lines were tested at the baseline for CES1 expression and bioenergetic parameters by qRT-PCR, Western blot (WB) and Seahorse XFe96. Changes in CES1 levels, metabolic phenotype, autophagy flux and survival under energy stress conditions with or without specific CES1 inhibitor were evaluated by qRT-PCR, seahorse, WB and viability assay. We found that elevated CES1 expression correlates with worse prognosis in OC patients. Accordingly, we showed that CES1 was basally expressed only in the cell lines EFO21 and OV-56 established from metastatic tumours. Although OVCA-429 and ES2 cell lines established from primary tumours were lacking basal CES1 expression, all OC lines expressed CES1 when cultured under energy stress, suggesting that CES1 could be relevant for their adaptation to harsh metabolic environment. Indeed, pharmacological CES1 blockade by commercially available GR-148672X inhibitor impaired bioenergetic parameters and blocked autophagy flux, thus resulting in significant cell death of all the analysed OC lines. Notably, inhibition of CES1 signalling under energy stress conditions was also able to kill carboplatin-resistant OC cells. These data underscore the clinical relevance of this enzyme in OC and suggest that CES1 could be a potential druggable target, especially in chemotherapy-refractory subsets.

P22.

IMPACT OF OUTER MEMBRANE VESICLES DERIVED FROM KLEBSIELLA PNEUMONIAE ON MIRNA EXPRESSION PROFILE IN HUMAN BRONCHIAL EPITHELIAL CELLS

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Introduction: Klebsiella pneumoniae (K. pneumoniae) is an opportunistic pathogen, leading cause of community- and hospital-acquired infections. K. pneumoniae employs several virulence factors that allow for adhesion and invasion of host cells. Moreover, it rapidly gains resistance to antibiotics, limiting therapeutic options for treating infections. Due to the significant public health impact, better understanding of virulence mechanisms is essential to develop new antibiotics against K. pneumoniae infections. Like any Gram-negative bacterium, K. pneumoniae constitutively releases outer membrane vesicles (0MVs). These vesicles act as vectors of virulence determinants, inducing cell damage. Our previous investigations demonstrated a variation of gene expression in response to treatment with OMVs. This alteration could be induced by microRNA (miRNA). There is little evidence for changes in miRNA expression in human bronchial epithelial cells after treatment with OMVs. Therefore, the aim of the present study was to evaluate the changes in miRNA expression after treatment with OMV produced by the standard K. pneumoniae strain and isolated in the field.
Materials and Methods: OMVs from standard K. pneumoniae strains and clinical isolates were purified from standard K. pneumoniae cultures and clinical isolates by serial ultrafiltrations and ultracentrifugations. The vesicles were quantized using a Bradford test. Thereafter, their diameter and polydispersity index were evaluated by Dynamic Light Scattering (DLS). The BEAS-2B cell line was treated with 5µg/mL of OMV for 6 hours and miRNA extractions were performed on treated and non-treating cells. MiRNA expression profiles were assessed by the 384-well TaqMan Human MicroRNA array. TargetScan, DIANA-microT-CDS and miRTarBase were used to predict the target genes of each miRNA. Metascape software was used for Gene Ontology enrichment analysis. The transcript levels of four miRNAs were validated by RT-qPCR.
Results: DLS analysis showed that the OMV of K. pneumoniae ATCC 10031 exhibited a diameter of 273.3 ± 1.3 nm and was characterized by a slightly heterogeneous size distribution. Diameters of 427.1 ± 0.9 nm and 483.3 ± 1.7 nm and greater heterogeneity were found for multisensitive (MS) and carbapenemase-producing (KPC) strains. Moreover, greater vesicular production was found in clinical isolates compared to the standard strain. Microarray and RT-qPCR analysis revealed dysregulation of miR-223, has miR-21, hsa-miR-25 and hsa-let-7g. Prediction of target genes showed their role in the host immune response, involving NF κB (miR-223), TLR4 (hsa-miR-21), cytokines (hsa-miR-25) and IL-6 (hsa-let-7g miRNA) signaling pathways.
Discussion and Conclusions: Our findings highlighted a significant role of OMVs in the inflammatory response via miRNAs regulation.

P23.

ROLE OF MST1 IN THE DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION AND VASCULAR DAMAGE IN RESPONSE TO METABOLIC STRESS

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The Hippo pathway is a master regulator of cell survival and growth. Preclinical evidence showed that the in vivo overexpression of the mammalian sterile 20-like kinase 1 (MST1), a key component of the Hippo pathway, leads to cardiomyocytes apoptosis, dilated cardiomyopathy and hearth failure. However, the potential role of the Hippo pathway in the development of endothelial and vascular damage in response to metabolic disorders is still unclear. We evaluated MST1 activity in human umbilical vein endothelial cells (HUVECs) treated with high glucose or oxidized low density lipoproteins (oxLDL). For overexpression and inhibition studies, adenoviruses overexpressing either a wild type (AD-MST1) or a dominant negative form of MST1 (AD-DN-MST1) were used. Angiogenesis, cell survival, apoptosis, oxidative stress, RAC1 activity and nitric oxide (NO) metabolism were assessed in these conditions. For vascular reactivity experiments, mesenteric arteries isolated from mice (C57BL/6) were subjected to high glucose or to oxLDL treatment either in the presence or in the absence of MST1 inhibition. We firstly observed that MST1 is activated in response to hyperglycaemia or to oxLDL in HUVECs. We also found that MST1 overexpression induces apoptosis ($p<0.05$) and impairs angiogenesis ($p<0.05$) and nitric oxide (NO) metabolism ($p<0.001$). Inhibition of MST1 rescues the deleterious effects of both high glucose and oxLDL treatment. It also improves the function of mesenteric arteries exposed to metabolic stress ex vivo ($p<0.05$). Mechanistically, we demonstrated that MST1 overexpression promotes reactive oxygen species (ROS) production ($p<0.001$) and RAC1-NOX2 activation. On the other hand, MST1 inhibition reduces ROS ($p<0.05$ vs high glucose; $pp<0.001$ vs oxLDL) and RAC1-NOX2 activation in stressed HUVECs. We finally found that RAC1 inhibition rescues the deleterious effects induced by MST1 overexpression. Our data suggest a fundamental role of the Hippo pathway in endothelial/vascular dysfunction induced by metabolic stress. Inhibition of MST1 may be considered as a potential strategy for the prevention of cardiovascular diseases related to metabolic disorders, such as obesity and diabetes.

P24.

MUTATIONAL ANALYSIS OF GENES IN CANCER PATIENTS FROM THE SICILIAN POPULATION

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Despite many significant advances in the early detection and treatment of localized tumors, cancer still ranks as the second leading cause of death worldwide, due to the unavailability of efficacious treatments against the most aggressive and metastatic diseases. To date, the choice of treatment plan for cancer patients is made on the basis of the histological analysis of surgical resections or biopsies. Thus, there is an urgent need to develop new effective therapeutic strategies. With this in mind, the improvement of "personalized" medicine is one of the primary goals of translational oncology. Next generation sequencing (NGS) technology has allowed not only to analyze multiple genes simultaneously in a sample, but also several tumor samples, reducing data acquisition time. This has revolutionized the management of cancer patient, as the criteria for treatment choice could be based on the mutational profile of the individual patient. Furthermore, the possibility to simultaneously characterize patient's somatic and germline DNA, identifying the presence of hereditary alterations on genes predisposing to the tumor, will allow a more complete clinical classification, with active surveillance plans even for family members who have not yet developed a tumor pathology. The aim of our study is to investigate the incidence and distribution of germline and somatic alterations in a cohort of about 150 cancer patients (pancreatic, breast and colorectal) from Sicily evaluating their associations with specific tumor features and predicting treatment effects and outcomes. The Oncomine focus DNA/RNA panel and a DNA custom panel, including 172 risk genes, 295 altered genes in tumors and 196pharmacogenetic variants, were used to perform NGS in retrospective and prospective cancer patients, respectively. The possibility of carrying out a broad spectrum (comprehensive) diagnostic test will improve the prognosis and the quality of life of cancer patients, thanks to a personalized therapeutic approach aimed at targeting only the tumor tissues, preserving the healthy ones. The results of the project will be translated in the validation of a service that can be usefully used in clinical practice. In the future, NGS mutation status screening could potentially help clinician to make the appropriate treatment decision.

P25.

SCD14-ST and New Generation Inflammatory Biomarkers in the Prediction of COVID-19 Outcome

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Since no definitive cure for COVID-19 is available so far, one of the challenges against the disease is understanding the clinical features and the laboratory inflammatory markers that can differentiate among different severity grades of the disease. The aim of the present study is a comprehensive and longitudinal evaluation of SCD14-ST and other new inflammatory markers, as well as cytokine storm molecules and current inflammatory parameters, in order to define a panel of biomarkers that could be useful for a better prognostic prediction of COVID-19 mortality. SCD14-ST, as well as the inflammatory markers IL-6, IL-10, SuPAR and sRAGE, were measured in plasma-EDTA of ICU COVID-19 positive patients. In this longitudinal study, SCD14-ST resulted significantly higher in patients who eventually died compared to those who were discharged from the ICU. The results suggest that the new infection biomarker SCD14-ST, in addition to new generation inflammatory biomarkers, such as SuPAR, sRAGE and the cytokines IL-6 and IL-10, can be a useful prognostic tool associated with canonical inflammatory parameters, such as CRP, to predict SARS-CoV-2 outcome in ICU patients.

P26.

SALIVARY NON-ESTERIFIED FATTY ACIDS AS NEW POTENTIAL BIOMARKERS FOR LUNG FUNCTION EVALUATION IN CYSTIC FIBROSIS

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A recent literature related lipidomic profile to cystic fibrosis (CF), revealing a complex crosstalk between lipids and inflammatory/immune pathways. CF is a multisystemic disease characterized by a chronic inflammation involving most tissue and organs among which the lung with the impairment of airway secretions. Clinical monitoring of lung disease in CF patients requires the invasive collection of bronchoalveolar lavage fluid. The analysis of salivary biomarkers could represent a non-invasive alternative approach.
We analyzed cholesterol, non-esterified fatty acids (NEFA) and total fatty acids (TFA) together with inflammation markers, i.e., interleukin-6 (IL-6), IL-8, and tumor necrosis factor alpha (TNF-α) in resting saliva samples from CF patients (n = 69) and healthy subjects (n = 50), relating to lung disease severity and sinonasal complications, i.e., inferior turbinate hypertrophy (NTH) and/or nasal polyposis (NP).
CF patients showed significantly higher levels of salivary IL-6, IL-8, and TNF-α than healthy subjects. Among CF patients, we found that IL-6 and IL-8 increased in NTH (acute phase of sinonasal disease), while TNF-α decreased in severe pulmonary disease and NP (chronic phase of sinonasal disease). Moreover, CF patients showed higher salivary levels of cholesterol, total NEFA, Unsaturated/Saturated (U/S) NEFA ratio than controls, while the U/S TFA ratio was significantly lower in CF patients, suggesting an altered activity of lingual lipase in CF, according with the literature [M. Roulet et al. Pediatr Res 1980;14:1360-1362]. Interestingly, U/S NEFA ratio was positively correlated with IL-6 and lung disease severity and may represent a prognostic marker in CF.

P27.

INSIGHTS ON THE THERAPEUTIC POTENTIAL OF ADIPOSE-DERIVED STEM CELLS AND THEIR SECRETOME: EPIGENETIC/MOLECULAR APPROACHES FOR FUNCTIONAL ENHANCING AND 3D MODELS TO IDENTIFY OPTIMIZED PATIENT TAILORED STRATEGIES

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Adipose-derived mesenchymal stem cells (ASCs) represent a valid therapeutic option for clinical application in several diseases, due to their ability to repair damaged tissues and to mitigate the inflammatory/immune response. We investigated the effects of the demethylating agent 5-azacytidine on several biological processes of ASCs (proliferation, migration, adipogenic differentiation and senescence). Understanding the underlying mechanisms regulating ASC biology represent a chance to modulate their in vitro characteristics and differentiation potential to improve their features for cell-based clinical approaches. ASCs exert their therapeutic action especially through their paracrine activity, by secreting soluble factors and extra-vesicles collectively known as secretome. We focused on ASCs secretome as an alternative therapeutic tool to cell therapies. In fact, by modifying the composition of the secretome we are also able to modulate the therapeutic effects of ASCs on target cells. Transfection with microRNAs, such as miR-125b, represents a feasible strategy to potentiate ASCs secretome, leading also to a modulation of its cytokines composition. Epicardial adipose tissue (EAT), the fat depot that directly surrounds the heart, has sparked interest due to its endocrine role, establishing a paracrine crosstalk with the underlying myocardium. EAT features have been correlated to the onset and progression of cardiovascular diseases (CVDs). EAT also represents a source of ASCs, known as epicardial ASCs (e-ASCs), with a high cardiomyogenic potential and marked pro-angiogenic and immunomodulatory abilities. However, little is known about the e-ASCs contribution to the milieu affecting cardiac function and the repair process associated with CVDs. We are characterizing e-ASCs differentiation potential and paracrine effects and analyzing gene modulation in the cardiovascular inflammation niche to shed light on e-ASCs therapeutic activity carried out through their secretome. Microfluidic chips exhibiting cardiomyocytes associated with microvascular endothelial cells and human cardiac fibroblasts allow us to study cardiac pathophysiological mechanisms under different microenvironmental and systemic constraints. A 3D heart-on-a-chip model summarizing the main aspects of a three-dimensional structure and integrating the different microenvironmental variables will allow us to characterize the potential impact of e-ASCs and their secretome on cardiovascular diseases, and to find novel strategies to modulate the biological features of cardiomyocytes and their microenvironment.

P28.

ROLE OF FGFR2C/PKCΕ SIGNALING IN THE CONTROL OF MCL-1-MEDIATED CELL SURVIVAL AND INVASION IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS

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The pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy characterized by KRAS activating mutations and aggressive phenotype. Since we recently identified the high aberrant expression of the mesenchymal isoform of FGFR2 (FGFR2c) and its downstream PKCε signaling as responsible for the enhancement of EMT profile, here we investigated their involvement in the establishment of additional tumorigenic features. Using stable protein depletion by shRNA in PDAC cell lines expressing divergent levels of the FGFR2c, we found that FGFR2c/PKCε axis is responsible for FGF2-mediated cell invasion and for anchorage-independent growth, while *in vitro* clonogenic assays, coupled to cleaved PARP1 check by Western blot, highlighted its involvement in cell viability. Finally, monitoring of MCL-1 expression and SRC phosphorylation suggested that a FGFR2c/PKCε-mediated control of cell migration/invasion via MCL-1/SRC-dependent reorganization of actin cytoskeleton. The identification of PKCε as hub molecule downstream FGFR2c at the crossroad of signaling networks governing the main malignant tumor hallmarks could represent an important advance towards innovative target therapies overcoming RAS.

P29.

IS BIPOLAR DISORDER THE CONSEQUENCE OF A GENETIC WEAKNESS OR NOT HAVING CORRECTLY USED A POTENTIAL ADAPTIVE CONDITION?

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Introduction: It has been hypothesized that specific personality traits associated with a high risk of psychopathology, specifically at high risk of bipolar disorder, could, under defined conditions, produce adaptive behaviors. This work, according of this approach, wants to verify whether a genetic feature associated with bipolar disorder can be found in people without bipolar disorder but with characteristics of hyperactivity and exploration.
Materials and methods: The target population included healthy elderly people living in an urban area recruited for a previous study on physical exercise, while the control group involved patients with a formal diagnosis of bipolar spectrum disorders. Both groups (61 participants in total) were aged 60 or over 60 years. The genetic procedure consisted of blood sampling, DNA extraction, real-time PCR together with FRET probes, and SANGER method sequencing. The genetic variant RS1006737 of CACNA1C was analysed.
Results: People with hyperactivity and without bipolar disorder (+H-BP) are homogeneous with people with bipolar disorder (+BP) regarding the frequency of the genetic variant RS1006737 (OR=0.79, CI 95%0.21-2.95), but don't with people without hyperactivity and without the bipolar disorder (-H-BP)(OR=4.75, CI95% 1.19-18.91). If the group with hyperactivity and without bipolar disorder is added to the group with bipolar disorder, the set of the two groups has a frequency of the variant RS1006737 clearly higher than the group without hyperactivity and without the bipolar disorder (OR=4.25, CI95%1.24-14.4).
Conclusions: Consider the genetic characteristics associated with bipolar disorder not as an "aberrant condition" would open the way to a new approach to supporting drug therapy, in which the rediscovery of the adaptive potential would be a central point in the psychosocial approach for the individual with bipolar disorders. For these reasons, it is important that future research can confirm the results of our study.
Keywords: Bipolar disorder, hyperactivity evolutionary perspective, RS1006737, CACNA1C

P30.

FUNCTIONAL SCREENINGS CONVERGE ON MDM2 AS A MODULATOR OF PROTEASOME INHIBITOR RESISTANCE IN MULTIPLE MYELOMA

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Multiple Myeloma (MM) is a genetically heterogeneous plasma-cell malignancy. Despite the availability of therapeutic regimens, including proteasome inhibitors (PIs) combinations with immunomodulatory drugs and monoclonal antibodies, drug resistance remains a major obstacle to MM complete eradication. In this study, we integrated complementary functional approaches to reveal synthetic lethal interactions that could be exploited therapeutically. Using a genome-wide CRISPR activation screen, the Mouse Double Minute 2 homolog (MDM2) gene emerged as a potential modulator of carfilzomib (CFZ) resistance. In addition, NVP-CGM097, an MDM2 selective inhibitor, was identified to synergize with CFZ treatment in a pharmacological screening. Our experiments demonstrate that MDM2 transactivation enhances MM cells recovery after cytotoxic CFZ treatment and confers selective advantage in cell competition assays under PI treatment. Furthermore, combination of CFZ with NVP-CGM097 significantly increases MM cell death by upregulating p53 levels and activating apoptosis. All together, our results suggest that MDM2 may be instrumental in driving CFZ resistance, and thus reinforce the interest in further exploring the therapeutic potentials of the suggested combination to overcome PI resistance in MM and in other neoplasms. Since NVP-CGM097 is currently under clinical trial for solid tumors, these results could support the possibility to translate its use for hematological malignancies.

P31.

NON-INVASIVE BRAIN STIMULATION TECHNIQUES IN SLEEP DISORDERS: FROM NEUROPHYSIOLOGY TO NEUROMODULATION

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Recently, there has been a remarkable understanding of the pathophysiology of some sleep disorders, such as Restless Legs Syndrome (RLS) and REM sleep Behavior Disorder (RBD), especially regarding the identification of early electrophysiological markers and the possibility to modulate them through non-invasive brain stimulation. Neurophysiological data in RLS converge on the concept of a complex sensory-motor disorder in which cortical, subcortical, spinal, and peripheral nerve generators are all involved in a dysfunctional network, eventually resulting in enhanced excitability and decreased inhibition. Although the spinal component has dominated in neurophysiological assessment, possibly due to better accessibility compared to other locations, multiple mechanisms, such as reduced central inhibition and abnormal peripheral nerve functioning, seem to equally contribute to the RLS pathogenesis. Translationally, the understanding of the complex interactions between central and peripheral neuronal circuits in generating the sensory-motor symptoms of RLS is mandatory for a better diagnostic refinement and innovative therapeutic support. Further evidence of altered central and peripheral excitability in RLS is provided by the efficacy of some non-pharmacological tools, such as repetitive transcranial magnetic stimulation, transcranial direct current stimulation (DCS), and transcutaneous spinal DCS, in transiently modulating neural excitability, thereby extending the therapeutic arsenal. In RBD, the pathogenesis of isolated form, i.e., before its phenoconversion in Parkinson's disease or other neurodegenerative disorders, still remains a matter of debate. Evidence of an involvement of several brainstem structures comes from several animal models and some neuropathological observations. However, both in vivo and in vitro studies have also examined how motor neurons are controlled during REM sleep, leading to the identification of an upstream brain circuitry that ultimately may trigger motor atonia. In this context, diagnostic techniques that electrophysiologically explore and measure other alterations beyond the brainstem in RBD are relevant to determine whether a prodromic neurodegenerative disorder underlies this condition might occurr at the stage of isolated RBD. This is in line with recent basic and clinical evidences that support the model of retrograde influence of the motor cortex on brainstem nuclei and the view of RBD as a widespread network disorder that goes far beyond the brainstem and the acetylcholine only.

P32.

FUNCTIONAL EFFECTS OF HUMAN LUTEINIZING HORMONE/CHORIOGONADOTROPIN RECEPTOR (LHCGR) AND G PROTEIN-COUPLED ESTROGEN RECEPTOR (GPER) HETEROMERS IN VITRO.

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Human luteinizing hormone (LH)/choriogonadotropin (hCG) receptor (LHCGR) and the G protein-coupled estrogen receptor (GPER) are co-expressed in ovarian granulosa cells, where they regulate follicle growth and oocyte maturation. In previous studies we demonstrated that GPER forms heteromeric complexes with the follicle-stimulating hormone (FSH) receptor (FSHR), which is structurally similar to LHCGR. FSHR-GPER hetromerization mediates the reprogramming of FSH-induced death signals linked to cAMP increase into activation of AKT-dependent survival signals. In this research, we evaluated whether GPER interacts with LHCGR modulating LH/hCG-dependent intracellular signalling.

LHCGR-GPER heteromers were evaluated in transiently transfected HEK293 cells co-expressing the two biosensor- or FLAG/HA-tagged receptors and signals were collected by bioluminescence resonance energy transfer (BRET) and photo-activated localization microscopy using photoactivatable dyes (PD-PALM), which allowed the identification of specific LHCGR-GPER interactions. GPER modulation of LHCGR-mediated pathways was evaluated by measuring LHCGR/G proteins coupling displacement and intracellular Ca2+ increase by BRET, cAMP and inositol monophosphate (IP1) accumulation induced by LH and hCG treatment by homogeneous time-resolved fluorescence (HTRF) and activation of reporter gene transcription regulated by nuclear factor of activated T-cells (*NFAT*) and cAMP response elements (*CRE*) promoters. Data from 4 to 6 experiments were statistically analysed by non-linear regression or Kruskal-Wallis test and Dunn's post-hoc test ($p<0.05$), as appropriate.

PD-PALM analysis revealed that about 20% of FLAG-GPER and HA-LHCGR expressed on the surface form heteromers, and results were confirmed by BRET detecting yFP-GPER and rluc-LHCGR interaction ($r2= 0.91$; $n=6$). Interestingly, BRET measurements showed that GPER displaced the LHCGR/Gαq coupling, resulting in the depletion of the hCG-induced intracellular Ca2+ increase under GPER and LHCGR co-expression (AUC LHCGR= 5169 ± 506 *vs* AUC LHCGR+GPER= 3621 ± 277; $p<0.05$; $n=6$). These results reflect the failure of LH/hCG treatment in inducing IP1 accumulation in LHCGR/GPER co-expressing cells (LH=2,72±0.49 nM; hCG=10,89±2,55 nM; $p\geq0.05$; $n=4$), oppositely to what detected in cells expressing the LHCGR alone (LH=31,48±5,45 nM; hCG=52,77±4,31 nM; $p<0.05$; $n=4$), and in activating downstream IP1/Ca2+ target gene reporter, which transcription is regulated by *NFAT* promoter (LHCGR LH=2.4±0.3 *vs* LHCGR+GPER LH=0.6±0.1; LHCGR hCG=2.5±0.3 *vs* LHCGR+GPER hCG=0.7±0.1; $n=5$; $p<0.05$). However, GPER-LHCGR complexes have no impact on LH/hCG-induced cAMP production ($p>0.05$) and on cAMP/protein kinase A (PKA) pathway activation. Control experiments were performed using a biosensor-tagged mutant GPER (GPERmut) unable to form heteromers with LHCGR, in transfected HEK293 cells. PD-PALM and BRET data revealed the absence of GPERmut-LHCGR complexes and the lack of heteromeric formations ($r2=0.05$; $n=6$). As expected, in GPERmut/LHCGR co-expressing cells, IP1 accumulation and gene transcription occur upon LH/hCG treatment (LHCGR *vs* LHCGR+GPER; $p>0.05$; $n=5$).

In conclusion, GPER and LHCGR interact in the cell surface modulating the Gq protein-mediated signalling cascades. LHCGR/GPER heteromers inhibit LH/hCG-dependent intracellular Ca2+ increase, IP1 accumulation and *NFAT* promoter activity, suggesting a possible role in regulating ovarian physiology.

P33.

PROTEOMIC ANALYSIS REVEALED SALL4 AS A SUBSTRATE OF CRL3REN COMPLEX AND A NEW THERAPEUTIC TARGET FOR SONIC HEDGEHOG-DEPENDENT MEDULLOBLASTOMA.

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Medulloblastoma (MB) is the most common pediatric brain tumor that arises from alterations in cerebellum development. The Sonic Hedgehog variant (SHH-MB) is the best genetically characterized, however the molecular mechanism responsible for its pathogenesis are not fully understood. Here, we show that the stemness regulator and pro-oncogenic Spalt-like transcriptional factor 4 (SALL4) is re-activated in mouse SHH-MB models, and its high expression correlates with worse overall survival in SHH-MB patients. Proteomic analysis revealed SALL4 as a new interactor of REN/KCTD11 (here REN), the substrate specific adaptor of the Cullin3-RING ubiquitin ligase complex (CRL3REN) and tumor suppressor lost in ~30% of human SHH-MBs. We demonstrate that CRL3REN induces SALL4 polyubiquitylation and proteasome-mediated degradation. Interestingly, SALL4 binds GLI1 and works in complex with HDAC1 to promote GLI1 deacetylation and to induce its transcriptional activity. Of note, genetic depletion of SALL4 or its pharmacological inhibition mediated by the immunomodulatory imide drug (IMiD) thalidomide significantly inhibits SHH-MB tumor growth both in murine and Patient-Derived Xenograft models. Our findings identify SALL4 as a novel CRL3REN substrate and promising therapeutic target for SHH-dependent cancer.

P34.

BLOOD-DERIVED CYTOLOGICAL PREPARATION OF CANCER WITH CHARACTEX PROTOCOL

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Liquid biopsy, especially when performed by the isolation, expansion, and examination of Circulating Tumor Cells (CTCs) from peripheral blood, has become an innovative and transforming diagnostic tool in Clinical Oncology. The use of liquid biopsy to detect post-surgical and/or post-treatment minimal residual disease to predict cancer recurrence and real-time treatment response has given more space to a personalized approach to the cancer patient. This progress has led many scientists to wonder whether liquid biopsy might ultimately substitute for tumor biopsy. In this context, the retrospective observational project known as CHARACTEX, based on cytologic screening and evaluation of the proliferation rate of CTCs has permitted to state that it is possible to exploit cytologic samples , through short-term culture and expansion of CTCs on chamber slides, can reduce the distance between traditional cytopathologic samples and those obtained by the blood. Charactex's method is based initially on a gradient-sedimentation technique, that impoverishes without completely depriving the obtained sample from the hematological cells, followed by short-term (14 days) in vitro culture and expansion of the cell population possessing proliferative advantage The examination of the expanded cell population obtained by this method is very rewarding both for the pathologist, who can assess multiple variables fitting with criteria which are consistent to the known primary tumor, like immunocytochemistry, flow-cytometry of several parameters molecule pathology on cell suspension and cell blocks obtained from them.

P35.

ROLE OF ANGIOTENSIN II IN MAINTAINING GLIOBLASTOMA STEMNESS THROUGH THE CROSSTALK BETWEEN PI3K/AKT AND HEDGEHOG PATHWAY.

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Glioblastoma multiforme (GBM) is the most common and aggressive primary intracranial malignancy, causing 3–4% of all cancer-related death. Currently, the standard treatments for GBM include surgical resection in combination with radiotherapy and chemotherapy with Temozolomide. However, despite enormous efforts in multimodal treatment approaches, the prognosis of glioblastoma remains very poor, with a median overall survival of less than 15 months after diagnosis. GBM is characterized by extensive phenotypic, morphological, and cellular heterogeneity that is thought to be maintained by a population of transformed stem-like cells referred to as glioma stem cells (GSCs). It has been reported that GSCs contribute to tumor initiation, invasion, recurrence, and resistance to therapy due to their self-renewal ability and multi-lineage differentiation potential. Thus, the identification of novel molecular mechanisms that sustain the stem-like properties of glioblastoma cells will be important for therapeutic purpose.
Recently, the known canonical hallmarks of cancer were connected with several biochemical pathways, including those of the renin-angiotensin system (RAS). The discovery of the RAS system and receptor in GBM mostly involved were those modulating cellular matrix reorganization. For instance, according to transcriptional network analysis, the positive expression of Angiotensin II Receptor Type 1 (AGTR1) and Angiotensin II Receptor Type 2 (AGTR2) have been correlated with different sets of hub genes involved in protumoral function in the C6 glioma cell line, suggesting that both Angiotensin II (Ang II) receptors may represent potential therapeutical targets. It has been reported that Ang II stimulates estradiol secretion from human placental explant in a dose-time dependent fashion together with a decrease of the total levels of estrogen precursors such as androstenedione and testosterone, addressing how the action of Ang II relies on its stimulatory effect on aromatization step. This effect was blocked by the selective AGTR1 antagonist Losartan. Accordingly, it has also been reported in cattle that short-term exposure to Ang II affects granulosa cell genes involved in estradiol secretion, such as cytochrome P450 aromatase (CYP19) mRNA, determining granulosa cell proliferation, differentiation, and consequently follicle growth.
Recently, we have demonstrated that Ang II/AGTR1 signalling enhances local estrogen production in GBM through the induced upregulation of aromatase gene expression. In the present study we observed that enhanced local estrogen production, trough non genomic action mediated by estrogen membrane receptor α contributes to the enhanced activation to PI3K/AKT pathways. Leading to the increased expression of GL1 which is nuclear mediator of the Hedgehog pathway, involved in Glioblastoma cell stemness.
Our data show that anti-estrogen treatment in U87MG, upon AngII treatment, reverses the increase of PI3K / AKT activity, concomitantly with a down-regulation of GL1 expression leading to a strong reduction of neurospheres cells production.
Thus its is reasonable to conclude that Ang II/AGTR1 - estrogen signalling - PI3K/AKT–GL1 axis, is evolved in the Glioblastoma stemness leading to its growth and progression.

P36.

THE NEXT GENERATION OF MODEL SYSTEMS OF HUMAN CARDIAC ORGANOIDS IN A DISH

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Organoids are tiny, self-organized, three-dimensional tissue cultures that are derived from the differentiation of stem cells. The growing interest in the use of organoids arises from their ability to mimic the biology and physiology of specific tissue structures in vitro. Organoids indeed represent promising systems for the in vitro modeling of tissue morphogenesis and organogenesis, regenerative medicine and tissue engineering, drug therapy testing, toxicology screening, and disease modeling. Although 2D cell cultures have been used for more than 50 years, even for their simplicity and low-cost maintenance, recent years have witnessed a steep rise in the availability of organoid model systems. Exploiting the ability of cells to re-aggregate and reconstruct the original architecture of an organ makes it possible to overcome many limitations of 2D cell culture systems. In vitro replication of the cellular micro-environment of a specific tissue leads to reproducing the molecular, biochemical, and biomechanical mechanisms that directly influence cell behavior and fate within that specific tissue. Lineage-specific self-organizing organoids have now been generated for many organs. Currently, growing cardiac organoid (cardioids) from pluripotent stem cells and cardiac stem/progenitor cells remains an open challenge due to the complexity of the spreading, differentiation, and migration of cardiac muscle and vascular layers. In particular, we generated cardiac organoids from Duchenne Muscular Dystrophy (DMD-COs) patient-derived induced pluripotent stem cell model that displayed DMD-related cardiomyopathy and disease progression phenotypes in long-term culture. DMD is an X-linked neuromuscular disease without a cure. DMD-COs showed a reduced initial proliferative capacity, a progressive loss of sarcoglycan and a high endoplasmic reticulum stress markers. Moreover, DMD-COs displayed an initial normal cardiac phenotype which progressively deteriorated and exhibited pro-fibrotic and pro-adipogenic phenotypes upon long-term culture, resembling pathologic events associated with DMD cardiomyopathy. We demonstrated the feasibility to develop a more complex, realistic and reliable in vitro 3D human cardiac-mimics to study DMD-related cardiomyopathies.

P37.

TUMOR DERIVED EXTRACELLULAR MATRIX ALLOWS THE CREATION OF A PAZIENT-PERSONALIZED IN VITRO TUMOR

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The extracellular matrix (ECM, a highly organized structure, represents the major structural component of the tumor microenvironment. The ECM is a non-cellular network of proteins providing to the tumor cells the first environment to proliferate within, to colonize and to migrate from. Indeed, the tumor is a very dynamic microenvironment, the study of the ECM structure is the first step not only to investigate the nature of the tumor scaffold but also to understand cellular, molecular, and immunologic mechanisms of tumor response and resistance. Several well-known biomaterials were proposed to mimic the 3D tumor scaffold. However, the cell adhesion to these biomaterials do not reflect the real tumor cells-microenvironment interactions due to the lack of the complex structure typical of the ECM tumor. To overcome these limitations, we proposed to recreate a 3D tumor structure using the decellularized matrix of a native tumor as a scaffold to be colonized by a variety of cell types. This could allow the study of the interactions between tumor cells, immune system, and tumor matrix and, at the same time, the development of a culture system for the screening of drugs for targeted therapeutic models. Murine B16f10 melanoma cells were expanded and injected subcutaneously to C57Black/6 mice to create a tumor. After two weeks we harvested the tumor and started the decellularization process (United States Patent 6743574B1.2004) using a cryotube placed in a tilling system (static system) and a dynamic system using a bioreactor (U-CUP). The decellularization process was followed by recolonization of the tumor matrix by cells (GFP-MDA and NHI3T3). After 7 days the re-cellularized tumors were recovered and a histological analysis was performed. The decellularization process removed all the cells from the tissue as shown by the absence of DAPI positivity in the tissue sections and it was not detrimental to the extracellular fibers, as confirmed by the trichrome staining and the presence of collagen type VI. No significant differences were observed with both decellularization methods. However, when the efficacy of the decellularized tissue as scaffolds for 3D culture was tested by different recellularization, we observed difference according to the different protocols adopted. After 7 days of culture, in static system the cell attachment was limited to the border of the scaffold while in dynamic condition the cells were more integrated into the decellularized matrix. In static decellularization condition by structural histological analysis we observed a collapse of the matrix. This was confirmed by SEM images, that showed fibres twisted on themselves generating a complex net, impenetrable from cells. The murine melanoma tissue is suitable for the creation of a 3D in vitro tumor microenvironment when, to recreate conditions favorable to cell penetration into the matrix, decellularization and recellularization are performed in a dynamic system. This method could allow to obtain a patient-personalized tumor, adequate for drug screening that will eventually drive to the more appropriate drug therapy for each patient.

P38.

ATR-FTIR IMAGING CHARACTERIZATION OF COLLAGEN IN HUMAN SOFT TISSUES AROUND A NEW LASER- TREATED HEALING ABUTMENTS: FROM BENCH-TOP TO CHAIR-SIDE

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One of the key factors for the osseointegration of dental implants is represented by the soft tissue barrier, whose properties are affected by the characteristic of the implant components. This study aims to investigate the structural organization and the macromolecular composition of collagen bundles in gingiva portions close to healing abutments (HA) with different surfaces, laser treated and machined. At this purpose, the characterizations were carried out by histological analysis and by a noninvasive and high-resolution analytical technique, the Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Three consent patients were enrolled in this study. For each patient a custom-made HA, with alternatively laser and machined treated surfaces, were inserted in the dental implant. After 8 weeks, a punch of gingiva was collected. Samples were fixed with PFA 4% solution and paraffin embedded and sections were stained with Masson's trichrome staining for histological analysis and then, air-dry for ATR-FTIR imaging analysis. Multivariate analysis was performed as statistical analysis. Histological results highlighted an abundant amount of proteins, mainly represented by collagen, in the tissue around the HA. To better elucidate composition, topographical distribution and structural organization, IR maps were performed, focusing on the following spectral regions: 3630-3130 cm-1 Amide A), 1760-1490 cm-1 (Amide I and II), 1300-1185 cm-1 (collagen). Near the machined surface, collagen bundles were organized in a parallel way respect to the HA, while a perpendicular disposition was shown adjacent to the laser one. IR maps were also submitted to Hierarchical Cluster Analysis (HCA) and for each cluster the average spectrum was calculated in the 1800 to 900 cm-1 spectral region and vector normalized. Multivariate analysis suggested the presence of different spectral population within the mapped areas. The obtained preliminary results demonstrate that the use of a laser treated transmucosal surface can improve the morphological organization of the peri-implant collagen, whose distribution appears similar to that in natural tooth. Further studies are needed to fully understand the role of the HA surface in order to improve the dental implant success in clinical practice.

P39.

MULTIDISCIPLINARY APPROACH FOR THE STUDY OF DENTAL ENAMEL AND PERSPECTIVES FOR ITS REGENERATION

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Enamel, formed by epithelium-derived ameloblasts, is the hardest tissue in the human body and covers the dental crown, playing an essential role in protecting teeth against mechanical and chemical actions, as well as pathological insults. Once lost or damaged, it does not maintain the capacity to spontaneously heal since neither dental epithelial stem cells nor ameloblasts are present in the crown of adult functional teeth. Therefore, it needs to be replaced by different biomaterials that can functionally and esthetically restore it. A multidisciplinary approach, based on the coupling of high-resolution analytical techniques, such as Raman MicroSpectroscopy, Scanning Electron Microscopy, Energy Dispersive X-ray Spectrometry, and Vickers MicroHardness have been exploited to: (1) provide reliable and complete information on the microstructure and the chemical/elemental composition of human enamel, (2) validate a new analytical approach for obtaining multiple information on the different areas of dental enamel, and (3) propose innovative nanomodified biomaterials and effective clinical protocol useful for its repair. Furthermore, recent advances in stem cell-based therapy and modern technological and biological platforms for replacement of injured and lost enamel will be described, since they represent an attractive strategy that complements traditional biomaterials. An experimental animal model will be presented for studying the cell biology of amelogenesis. The proposed method consists of the in vivo administration of dental epithelial stem cells (DESCs) at the apical end of continuously growing rodent incisor in order to trace the fate of DESCs and to explore their potential to acquire epithelial lineage in vivo. These studies will contribute to implement knowledge on the mechanism underlying the possible regeneration of fully functional enamel.

P40.

LUTEINIZING HORMONE (LH)/HUMAN CHORIOGONADOTROPIN (HCG) RECEPTOR (LHCGR) INTERNALIZATION IS REQUIRED TO MODULATE LH- AND HCG- SPECIFIC SIGNALS

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Luteinizing hormone (LH) and human choriogonadotropin (hCG) are two heterodimeric glycoprotein hormones supporting steroidogenesis and reproduction through binding the same receptor (LHCGR), expressed in gonadal cells. They act via hormone-specific G proteins- and β-arrestin-dependent signals cascade, before LHCGR internalization into endosomal vesicles regulated by various Rab GTPases. The maintenance of LHCGR within endosomes persists for about 10 minutes, after which it could be recycled or addressed to late endosomes for lysosome degradation. Previous in vitro studies demonstrated that LH preferentially activates proliferative signals, whereas hCG upregulates mainly the steroidogenic pathway. In this study, we evaluated how LHCGR internalization determines LH- and hCG-specific signals. To this purpose, transfected HEK293 cells overexpressing LHCGR and specific bioluminescence resonance energy transfer (BRET) biosensors were used, in the presence and in the absence of internalization blockade by Dynasore. LH- and hCG-induced LHCGR internalization was evaluated over 30 min, determining the interaction between receptor and endosomal specific markers RABGTPases (Rab) 5, 7 and 11, as well as β-arrestin 2. Ligand-specific receptor coupling to Gs, Gi and Gq protein, cAMP activation, extracellularly-regulated kinases 1 and 2 (ERK1/2) and intracellular Ca2+ increase were evaluated as well. Results were compared by Kruskal-Wallis test and Dunn's post-test ($p<0.05$; n=4-8). Interaction between LHCGR and molecules such as Rab5- and β-arrestin 2-, which are markers of internalization or localization into early endosomes, was activated preferentially by cell treatment with hCG than LH ($p<0.05$; n=4). Moreover, hCG had higher efficacy than LH in inducing Gs and Gq coupling to LHCGR ($p<0.05$; n=8). These data reflect the downstream, marked hCG-dependent activation of cAMP and intracellular Ca2+ increase ($p<0.05$; n=4), as two molecules involved in steroid synthesis. LH induced preferential LHCGR-Rab11 interaction, as a key event to recycle the receptor in the cell membrane ($p<0.05$; n=4). Moreover, LH was more effective than hCG in inducing LHCGR coupling to Gi protein ($p<0.05$; n=8), resulting in the phosphorylation of the proliferation-related ERK1/2 ($p<0.05$; n=4). Cell treatment by Dynasore inhibited the LH-induced Gi coupling to the receptor and the downstream ERK1/2 phosphorylation (pÑ0.05; n=4), while increased the recruitment of Rab7, indicating ligand-specific routing through the degradation pathway. We conclude that LHCGR internalization is fundamental to modulate Lhand hCG specific signals, impacting the downstream signaling cascades.

P41.

THE ROLE OF MIR 335-5P IN THE REDIFFERENTIATION OF PAPILLARY-THYROID CANCER ORGANOIDS

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The most frequent mutation in papillary thyroid cancer (PTC) is the p.V600E of the BRAF gene. This mutation leads to aberrant activation of the RAS / BRAF / MEK / ERK pathway and the consequent under-regulation of thyroid-specific genes, resulting in uncontrolled growth and de-differentiation of cancer cells. We analyzed the transcriptomics data produced by the TCGA project using a network medicine approach. We identified 227 switch genes, 63 of them were found to be targets of the same microRNA, the mir-335-5p. The role of this microRNA was then investigated using an in vitro study. We selected two primary lines and four immortalized lines of PTC, all of them harboring BRAF mutation, and showing reduced levels of miR-335-5p expression. Cells were cultured both in bi-dimensional and three-dimensional conditions, obtaining organoid structures. We established a protocol to maintain long-term culture organoids. The medium of organoids was supplemented with conditioned medium obtained from the bi-dimensional cell line. This modification enables culture of the organoid lines for up to 10 months. Even after long-term culture, the organoids retain the genetic and phenotypic characteristics of their tissue of origin. The expression of thyroid-specific genes and protein was analyzed after the transfection of synthetic microRNA. In addition, protein localization was investigated with immunofluorescence both in bidimensional cell cultures and organoids. The restoration of the expression of miR-335-5p increased the expression of thyroid specific-genes (TSHR, PAX8, and NIS) mRNAs, the same results were confirmed in the analysis of the protein expression. Moreover, using immunofluorescence NIS and TSHR increased the expression and localized in the membrane in the membrane of cell lines and organoids overexpressed the miR-335-5porganoids. The restoration of the intracellular levels of mir-335-5p could have a role in promoting the re-differentiation of thyroid tumors harboring BRAF mutation.

P42.

VIRTUAL REALITY FRONTIERS IN BIPOLAR DISORDERS: A RECOVERY ORIENTED COGNITIVE REHABILITATION TOOL

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Background: Bipolar disorders are one of the main causes of disease worldwide. Cognitive deficits are a fundamental component of bipolar disorder, they negatively impact the personal and social functioning. Cognitive remediation interventions are effective in the treatment of various psychosocial disorders, including bipolar disorder. The use of Virtual reality as a rehabilitation tool has produced scientific evidence in recent years. This study aims at evaluating the feasibility of the first Cognitive Remediation Virtual Reality Program for people with bipolar disorder.
Methods: Feasibility randomized controlled cross-over clinical study; we will randomize 50 people from the Consultation and Psychosomatic Psychiatry Center of the University Hospital of Cagliari (San Giovanni di Dio Civil Hospital) with a diagnosis of bipolar disorder. We propose a CR program in VR, 3 months with 2 weekly sessions, for the experimental group and a usual care program for the control group (psychiatric visit and/or psychotherapy).
Results: We observed a significant feasibility outcomes and also clinical outcomes (both cognitive and personal and social functioning); in particular in memory, attention, semantic fluency, depressive symptoms, alexithymia and in biological rhythms.
Conclusion: This RCT aims, with regards to it feasibility and design, to inform a confirmatory trial that evaluates the effectiveness of a VR CR program in psychiatric rehabilitation for the treatment of cognitive dysfunction in people with bipolar disorder. The results could have an impact on psychiatric rehabilitation research with a focus on improving the application of technologies for mental health.

P43.

CROSS-INTERACTION BETWEEN PITUITARY GLYCOPROTEIN HORMONE AND RECEPTORS IN THYROID CANCERS

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Thyroid cancer is the most common type of endocrine tumor and reaches the peak of incidence between the age of twenty and fifty years. Thyroid cancer has 4-fold higher prevalence in females than males, suggesting that estrogens and their receptors could be involved in the pathogenesis. Previous studies demonstrated allosteric interference operated by G protein-coupled estrogen receptor (GPER) to molecules structurally similar to the thyroid-stimulating hormone (TSH) receptor (TSHR). We hypothesize that GPER may interact with TSHR, modulating proliferative signals in thyroid cells.
Mechanistic experiments were performed on papillary thyroid carcinoma (K1), follicular thyroid epithelial (Nthy-ori 3-1) and COS7 (control) cell lines and confirmed in different types of primary tumor and non-tumor thyroid tissues. Co-localization and heteromer formation of TSHR and GPER were evaluated in healthy tissues, and papillary, follicular and anaplastic thyroid tumors by immunofluorescence and proximity ligation assay (PLA). We transfected cell lines with GPER- and/or TSHR-coding plasmids to evaluate effects on the TSH-induced activation of Gαs and Gαq protein-associated transduction pathways, typically related to TSHR. Cell lines were treated with 300 nM TSH and 730 pM of the estrogen estradiol (E2). Then, we measured intracellular levels of IP1 by homogeneous time resolved fluorescence (HTRF), as well as intracellular cAMP and calcium ion (Ca2+) increase by bioluminescence resonance energy transfer (BRET). Results were compared by Kruskall-Wallis test ($n=6$; $p<0.05$) and corrected by Dunn's post-hoc test.
We found TSHR and GPER co-expression and physical interaction in healthy, non-tumor thyroid follicles, by immunofluorescence and PLA. Surprisingly, no indications of GPER expression were found in papillary, follicular and anaplastic thyroid cancer histological sections. In all the cell lines, we found that GPER and TSHR co-expression resulted in the inactivation of TSH-mediated Gαq-mediated
pathway, inhibiting both IP1 production and Ca2+ increase. However, no effects were found on TSH/GαS-mediated cAMP production. Control experiments performed using the Gαq inhibitor YM-254890 (10 μM) and the PLC inhibitor U73122 (50 μM) revealed GPER-like effects on TSH-induced IP1 production. Cell treatment with E2 and GPER antagonist (5 μM; G15) had no effects, revealing that GPER acts regardless of ligands.
In conclusion, the absence of GPER/TSHR heteromers plays a key role in the pathogenesis of three thyroid tumors. GPER may allosterically induce the inhibition of Gαq-dependent proliferative intracellular pathways activated by TSH through its receptor. This data suggests that GPER may play a role in increasing the incidence of thyroid cancer in women.

P44.

ANTI-PROLIFERATIVE SELECTIVE ACTIVITY OF DIRECT COLD ATMOSPHERIC PLASMA IN COMBINATION WITH CAPE DERIVATIVES IN ORAL SQUAMOUS CELL CARCINOMA CELLS

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The emerging field of plasma medicine employs cold atmospheric plasma (CAP) devices for cancer removal and more recently as emerging non-invasive anti-cancer agents capable of improving the efficacy of traditional drugs. Caffeic acid phenethyl ester (CAPE) is one of the most extensively investigated active components of propolis due to its anti-oxidant, anti-inflammatory, anti-viral, anti-fungal, and anti-tumoral properties. Here, we investigated antiproliferative effects of two different CAPE derivatives - newly designed and synthesized: MB10 and MB14 - in combination with CAP in non-malignant oral cells (HGFs) and in oral squamous cell carcinoma (OSCC) cells (HSC-2, CAL-27, HOC-621). First, the IC50 was calculated by exposing the cells to CAPE, MB10, and MB14 concentrations ranging from 0 to 100 µM for 48 h. HSC-2 was the most sensitive cell line, showing very promising IC50 for CAPE (12.68 µM), MB 10 (35.13µM), and MB14 (15.41µM). IC50 for CAL27 were: CAPE: 18.54µM, MB10: 37.30µM and MB14: 16.32µM, whilst in HOC-621 the IC50 for CAPE, MB10, and MB14 were 6.98, 38.67, and 16.85, respectively. Then, for direct CAP treatment, cells were treated with CAP for 30 and 60 sec at a distance of 12 mm; immediately after the compounds were added at concentrations of 5, 10, and 20 µM. The influence of CAP treatment on cell viability alone or in combination with MB10 and MB14 was evaluated after 48h. All the cell lines were affected by the direct CAP treatment. Interestingly, the combination of CAP treatment with low doses (5-20 µM) of MB10 and MB14 enhanced the anti-proliferative effects with a slightly different sensitivity among the cell lines. In particular, 5µM MB14 combined with 30 sec CAP was the mildest treatment being effective in reducing CAL-27 and HOC-621 proliferation. More importantly, HGFs were unaffected by the combination of 30 sec CAP and 5 and 10 µM MB14. These data demonstrated that the combination of the two approaches was selective and synergy toward cancer cells, thus allowing the use of the mildest conditions for both CAP and compounds Overall, these new findings may open a window for a new therapeutic approach for the management of OSCC in the future.

P45.

THE EFFECTS OF COMPLEX MAGNETIC FIELDS ON CANDIDA ALBICANS AND HUMAN FIBROBLASTS

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OBJECTIVE OF THE STUDY: It has been widely demonstrated that the use of weak electromagnetic fields promote the proliferation, orientation and migration of osteoblast-like cells. Histological studies confirmed the effects of enhancing the activity of bone cells, activating remodeling of the alveolar bone. However, the biological effects of these devices depend on various parameters, such as the duration of application, the frequency, the intensity, the emission mode, the waveform. Recently, new emitters of complex magnetic fields (CMF) have been introduced on the market characterized by programs containing waves of different shapes, very low intensity of the order of micro-Tesla and different frequency. CMF were administered on planktonic *Candida Albicans* and human fibroblasts to assess their growth and adhesion capacity at 24h on resin and titanium discs.
MATERIALS AND METHODS: Different programs (Antibacterial, oxidative stress and the combination of them) of the CMF device were tested on *Candida Albicans*. At the end of each treatment, the samples were diluted and plated on Sabourad-Agar plates. Then they were incubated at 37 ° C and the number of colony forming units per milliliter CFU / ml, metabolic activity, live / dead, cell morphology, filamentation analysis and fibroblast cytotoxicity test were determined. The same programs were tested on human fibroblasts (HGF) to evaluate the cell viability by the MTS assay. Furthermore, the different groups of treated *Candida* were grown on titanium and resin discs, to evaluate their growth and adhesion capacity at 24h.
RESULTS: The MTS assay after 24 h of C.M.F.s exposure showed that all treatments were fully biocompatible and exerted no negative effects on the viability of HGF. The C.M.F.s reduced the number of cultivable planktonic *Candida albicans* vs. controls, independently by the treatment applied. In particular, the antibacterial program was associated with lower levels of CFUs. The quantification of the metabolic activity was significantly lower by using the oxidative stress program. Live/dead images showed that C.M.F.s significantly decreased the viability of *C. albicans*. C.M.F.s inhibited C. albicans virulence traits reducing hyphal morphogenesis, adhesion, and biofilm formation on titanium discs.
CONCLUSIONS: CMF has shown a remarkable antifungal action against *Candida albicans* without affecting the proliferation of fibroblasts. Therefore, it could represent a very useful device for treating and preventing candidiasis.

P46.

MACHINE LEARNING IDENTIFIES SEX-SPECIFIC IMMUNOLOGICAL FEATURES TO ORAL MICROBIOME IN PERIODONTITIS AND HEALTHY INDIVIDUALS

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Recent evidence suggests that sex-based differences in immune responses exist that are, at least in part, shaped by sex-specific host-microbial interactions and that can impact on the individual susceptibility to a variety of diseases. We examined whether a sex-based dimorphism in the humoral immune response to the periodontal microbiota existed in a propensity score matched (PSM) cohort of adult men and women. One-to-one PSM was applied to male and female adult individuals (≥40 years) enrolled in the National Health and Nutrition Examination Survey (NHANES) III to obtain exact matches for age, race/ethnicity, periodontitis diagnosis and severity, hypertension history, diabetes, smoking habits, body mass index (BMI), and income between sexes. Participants underwent determination of serum antibodies to 21 periodontal microorganisms, as well as periodontal and biochemical evaluations. Machine learning (ML) approaches were applied to test whether specific antibodies could predict sex and if a sex-specific immunological phenotype could discriminate between healthy and periodontitis individuals. A total of 2724 exactly matched female and male participants (n. 1362/group) was included in the study (mean age: 57±12 years; 54.8% non-Hispanic Whites; 41.8% overweight; 55.9% smokers; 25.8% with periodontitis). Sex-based differences by periodontal health status were observed in terms to antibody titers to P.gingivalis, T.denticola, A.naeslundii, P.nigrescens, P.intermedia, C.ochracea, which differed between men and women only in healthy individuals, and T.forsythia and V.parvula, which differed based on sex only in individuals with periodontitis. These differences were driven by higher titers in men than in women, except for antibodies to A.naeslundii, where the opposite occurred. In ML, antibody titers to oral microbiome predicted sex with a sensitivity up to 67% and a specificity up to 55% during periodontitis, but not in healthy individuals. Age, BMI, and smoking did not substantially improve classification capacity. The humoral immune response to periodontal microbiota appears to be sex-specific, with different features in health and periodontal disease states. Future investigations are needed to clarify the clinical meaning of our findings, and whether a sexual dimorphism in the immune response carries prognostic implications in health and disease.

P47.

MAYER-ROKITANSKY-KÜSTER-HAUSER SYNDROME: NEW PERSPECTIVES ON MOLECULAR AETIOLOGY AND CLINICAL MANAGEMENT

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Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome is a rare and complex disease defined by congenital aplasia of the vagina and uterus in 46,XX women. The aetiopathogenesis of MRKH syndrome is still largely unknown. Since 2006, in collaboration with the gynaecology team of Policlinico Umberto I of Rome, we have performed vaginoplasty using a modified Abbé–McIndoe technique with autologous in vitro cultured vaginal tissue in patients with MRKH syndrome. We collected peripheral blood and vaginal dimple samples from patients to extract DNA and RNA for array-CGH, MLPA assays and molecular analyses. We explored the presence of pathogenic copy number variations (CNVs) in an Italian cohort of 36 unrelated MRKH patients and we identified aberrations in 25% of them. Interestingly, we highlighted a novel heterozygous microduplication at Xp22.33, containing the PRKX gene, and a novel duplication of a specific SHOX enhancer. To predict the potential significance of CNVs in MRKH pathogenesis, we provided a network analysis for protein coding genes found in the altered genomic regions. The computational network highlighted that the most relevant biological connections are related to the anatomical structure development. Furthermore, we investigated the role of selected candidate genes in the aetiopathogenesis of MRKH syndrome, with a focus on PRKX, which encodes for protein kinase X. Through RT-qPCR and PCA, we highlighted a phenotype-related expression pattern of PRKX, MUC1, HOXC8 and GREB1L in MRKH patients. By using an in vitro approach, we proved that PRKX ectopic overexpression in a cell model of vaginal keratinocytes promotes cell motility through EMT activation, a fundamental process in urogenital tract morphogenesis. Moreover, our findings showed that PRKX upregulation is able to affect transcriptional levels of HOX genes implicated in urinary and genital tract development. Our study identified the dysregulation of PRKX expression as one of the possible molecular mechanisms underlying MRKH syndrome. Currently, we are characterizing the microbiota of 20 MRKH patients, which successfully underwent vaginal reconstruction, and 20 healthy women, in order to study through microfluidic systems, the vaginal microenvironment and to develop pro/prebiotics-based strategies aimed at maintaining the correct balance of microorganisms for long-term health benefits of patients with MRKH.

P48.

A NOVEL HYBRID T:B CELL: A PARADIGM TO BE REWRITTEN OR AN ARTEFACT?

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Flow cytometry offers several advantages for the identification, enumeration, and characterization of cells. However, rare events can be the result of artefacts due to the presence of multiple fluorochromes that may increase the number of false positive events. Double-positive cells are one of the most representative examples of artefacts in flow cytometry staining and may appear also during dual-color immunophenotyping of lymphocytes. We and others found a novel hybrid T:B cell. This population consists of cells, combining antigens of B cells and T cells, in particular presenting both T and B cell receptors (TCR and BCR) on its surface. This finding is unexpected because cells of the adaptive immune system, during their early maturation, are primed to become either B cells or T cells, mature in different primary organs and rearrange different genes, to express either TCR or BCR. Thus, according to this dogma, lymphocytes can't have both receptors. This finding raised several conflicts, splitting the scientific community, believing or not, to this new population. Where is the truth? Single cell analysis, by investigating the transcriptomic profile of this T:B hybrid cell, might answer to this dilemma. If these findings will be confirmed, pairing surface protein data with transcripts one, a new paradigm in immunology needs to be rewritten.

P49.

RUOLO DI INNOVATIVI MEDICAL DEVICES NELLA RIGENERAZIONE DEITESSUTI: DAGLI STUDI IN VITRO ALLE APPLICAZIONI CLINICHE

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Obiettivo: La medicina rigenerativa rappresenta un approccio terapeutico finalizzato alla rigenerazione biologica del tessuto danneggiato, valendosi della capacità auto-rigenerativa intrinseca dei nostri tessuti, e non solo alla sua riparazione o sostituzione. A tal riguardo, l'obiettivo principale della relazione sarà quello di evidenziare l'efficacia in vitro ed in vivo dell'applicazione di nuovi protocolli di biostimolazione cellulare, sia in campo orale che dermatologico. In particolare, saranno presentati gli effetti dell'impiego di: (i) fotobiomodulazione (LED 630-830nm); (ii) un nuovo protocollo di biorivitalizzazione, che vede combinati l'utilizzo di un medical device a base di collagene e sostanze ancillari e la fotobiomodulazione (LED 640nm); (iii) un'innovativa tecnologia a plasma freddo alimentato ad aria, per trattamenti superficiali e di taglio.
Materiali e metodi: Gli effetti dell'irraggiamento con luce LED rossa sono stati valutati su colture primarie di fibroblasti e in diversi casi di ulcerazioni e ferite gravi. Inoltre, sono stati testati gli effetti del protocollo di "biorivitalizzazione collagenica" su colture primarie di fibroblasti, osteoblasti e cellule endoteliali, insieme ai case report dei trattamenti ambulatoriali. Infine, i vantaggi dell'utilizzo del bisturi a tecnologia "Airplasma", già dimostrati in vari ambiti, sono stati valutati sia per la disinfezione e la cura delle ferite, che come sostituto delle attuali tecniche di taglio in chirurgia orale.
Risultati e conclusioni: I risultati preliminari dimostrano che tali approcci innovativi promuovono la sintesi di nuovo collagene ed il rinnovamento strutturale, funzionale ed estetico del tessuto, favoriscono la migrazione cellulare e riducono l'infiammazione ed i tempi di guarigione delle ferite. In conclusione, sebbene siano necessari ulteriori studi, i nostri dati suggeriscono il potenziale impiego dei suddetti medical devices nella rigenerazione e riparazione dei tessuti.

P50.

SYNTHETIC RECOVERY OF ELECTROMECHANICAL PROPAGATION IN MYOCARDIAL INFARCTION VIA SILICON CARBIDE SEMICONDUCTIVE NANOWIRES

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Introduction: Myocardial infarction causes 7.3 million deaths worldwide, mostly from fibrillation that electrically originates from the damaged areas of the left ventricle (LV). Cardiac bypass graft and percutaneous coronary interventions allow reperfusion of the downstream tissue but do not counteract the electromechanical alteration originating from the less excitable infarct area (MI), thus blocking the conduction and favoring the reentrant electrical circuits.
Methods: In this work, we engineered and customize biocompatible silicon carbide semiconductive nanowires (SiC-NWs) that synthetically couple, via membrane nanobridge formations, cultured beating cardiomyocytes over distance. We inject SiC-NWs in the infarcted region of the heart and measure electromechanical coupling on the infarcted and border zone areas in the hours following the injection.
Results: We demonstrated restoring of physiological cell-cell electromechanical coupling and conductance, thereby permitting the synchronization of bioelectrical activity in otherwise uncoupled cells. Local in-situ multiple injections of SiC-NWs in the LV infarcted regions allow rapid reinstatement of impulse propagation across damaged areas and recover electrogram parameters, contractility compliance and conduction velocity.
Discussion: SiC-NWs biophysically regenerate physiological electrical conductance and synthetically restore electrograms (EGs) parameters and impulse propagation in MI regions within 5 h following the injection due to partial nano-integration with cardiomyocytes and myofibroblasts. We propose this nanomedical intervention as a strategy for rapidly reducing ventricular arrhythmia after acute MI.

P51.

EXPLOITING THE POTENTIALITY OF ADULT RENAL STEM/PROGENITOR CELLS: FROM THE ANTIAGING PROTEIN KLOTHO TO THE GENERATION OF ORGANOIDS.

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The complex system of human Adult Renal Stem/Progenitor Cells (ARPCs) and their crucial role in the homeostasis and regenerative processes in the adult kidneys have been recently discovered. Understanding factors regulating their behaviour is fundamental to develop treatments for renal injury and other diseases.
Latest studies showed that long no-coding RNA (lncRNAs) are essential to establish developmental patterning and maintain the stem cell pluri-potency networks and may be involved in cellular senescence processes.
For the first time, through a whole genome transcriptome screening analysis we studied lncRNA function in ARPCs. The lncRNA HOTAIR was found to be highly expressed in ARPCs. Through the CRISPR/Cas9 method, we generated ARPC lines knock-out for HOTAIR, demonstrating its role in regulating the self-renewal properties of ARPCs, supporting their proliferative capacity and limiting the apoptotic process. HOTAIR knock-out induced senescence of ARPCs and exerted its function through the epigenetic silencing of the cell cycle inhibitor p15, inducing the trimethylation of histone H3K27. Moreover, we found that HOTAIR influences ARPC ability to secrete high levels of the anti-aging protein, α-Klotho, which attenuates renal epithelial senescence and fibrosis by decreasing cell apoptosis and regulating cell cycle inhibitors.
The antiaging properties of ARPCs may be exploited also inducing them to generate renal organoids. For the first time we generated renal spheroids under 3D culture conditions without any stimulation (growth factors) starting from ARPCs isolated from urine patients. We showed that the spheroids express high levels of stem cell markers as CD133 and NANOG, functional and constitutional marker of ARPCs, and high levels of stem cell markers that normally are low or not at all expressed in ARPCs and are typical of embryonic stem cells: GATA-3, SSEA4, and Sox2. These data suggest that, in spheroids, ARPCs can dedifferentiate from multipotent to pluripotent cells. Furthermore, we showed that these organoids can give rise to long renal tubules.
In conclusion, HOTAIR influences ARPC ability to secrete high levels of α-Klotho that influences senescence in surrounding tissues and modulates, therefore, kidney aging. In the future, the secretome from spheroids generated by ARPCs may be used to revert the aging process of senescent cells.

P52.

LH INCREASES THE RESPONSE TO FSH IN GRANULOSA-LUTEIN CELLS FROM SUB/POOR-RESPONDER PATIENTS IN VITRO

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Introduction. Clinical studies suggested that FSH and LH co-treatment may be beneficial for the ovarian response of sub/poor-responders undergoing ovarian stimulation during assisted reproduction technique (ART).
Aim. We evaluated if LH addition to FSH treatment *in vitro* impacts the response of granulosa lutein cells collected from poor-, sub-, and normo-responder women undergoing medically assisted reproduction.
Methods. hGLC samples from 286 anonymous women undergoing oocyte retrieval for ART were collected from October 2017 to February 2021, and blindly purified, cultured, genotyped and treated *in vitro* by increasing concentrations of FSH (nM) ± 0.5 nM LH. cAMP and progesterone levels produced after 3 and 24 h, respectively, were measured. *In vitro* data were stratified *a posteriori*, according to the donors' ovarian response, into normo-, sub-, and poor-responder groups and statistically compared. The effects of LH addition to FSH were compared with those obtained by FSH alone in all the groups as well. Experiments were performed under the local Ethics Committee permission and written consent.
Results. hGLCs from normo-responders were shown to have higher sensitivity to FSH treatment than sub-/poor-responders *in vitro*. Equimolar FSH concentrations induced higher cAMP (about 2.5 to 4.2-fold), and progesterone *plateau* levels (1.2 to 2.1-fold), in cells from normo-responder women than those from sub-/poor-responders (ANOVA; $P < 0.05$). The addition of LH to the cell treatment significantly increased overall FSH efficacy, indicated by cAMP and progesterone levels, within all groups ($P > 0.05$). Interestingly, these *in vitro* endpoints, collected from the normo-
responder group treated with FSH alone, were similar to those obtained in the sub-/poor-responder group under FSH + LH treatment. No different allele frequencies and *FSHR* expression levels between groups were found, excluding genetics of gonadotropin and their receptors as a factor linked to the normo-, sub-, and poor-response.
Conclusions. This *in vitro* assay may describe the individual response to personalize ART stimulation protocol, according to the normo-, sub-, and poor-responder *status*. Moreover, it reflects results from clinical studies finding beneficial effects of FSH and LH co-treatment used during ovarian stimulation in certain ART patients and provides *in vitro* data supporting the personalized use of LH in assisted reproduction.

P53.

RISULTATI DELLO SCREENING GENETICO DI 270 PAZIENTI CON DIAGNOSI DI FEBBRE EREDITARIA RICORRENTE IN PUGLIA

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Le febbri ereditarie ricorrenti costituiscono un gruppo di malattie autoinfiammatorie ereditarie considerate disordini dell'immunità innata. I pazienti affetti presentano crisi febbrili ricorrenti associate a segni di infiammazione sistemica. La Febbre Mediterranea Familiare (FMF) rappresenta la più comune di queste sindromi. La FMF è stata considerata per lungo tempo una malattia considerata autosomica recessiva. Tuttavia, alcuni lavori pubblicati di recente suggeriscono che anche i portatori eterozigoti possano presentare i sintomi della malattia sebbene in forma più lieve. Nel nostro centro abbiamo sottoposto a testing genetico 270 pazienti e almeno una variante nel gene MEFV è risultata presente in poco più della metà dei pazienti testati (51,4%). 16/270 pazienti sono risultati portatori di variante in uno degli altri tre geni testati (NLRP3, MVK, TNFRSF1A). Delle 206 varianti identificate nel gene MEFV più della metà sono risultate R202Q (53.9%) Molto frequenti e spesso presenti in doppia eterozigosi sono risultate le varianti E148Q (12.1%) e R761H (8.7%). Abbiamo riscontrato che i sintomi presenti nei pazienti pugliesi tendono a essere più lievi rispetto a quelli presenti in pazienti di origine medio-orientale il che rende difficile l'applicazione dei criteri clinici di diagnosi adottati a livello internazionale. Inoltre la distribuzione di varianti MEFV osservata nella nostra coorte è significativamente diversa da quella presente in altri paesi del bacino mediterraneo. Infine, la gravità dei sintomi correlava con il numero di varianti identificate nei soggetti testati. E' possibile che la gravità dei sintomi dipenda da fattori ambientali non ancora identificati come peraltro già dimostrato in soggetti portatori di varianti patogenetiche di etnia turca ma residenti in Germania.

P54. MODELLI CELLULARI INNOVATIVI: IPOTESI APPLICATIVE TRASLAZIONALI NELLA MALATTIA DI PARKINSON.

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Obiettivo: Il morbo di Parkinson (PD) è una patologia neurodegenerativa caratterizzata dalla degenerazione di Neuroni Dopaminergici produttori di neuromelanina (DN) presenti nella substantia nigra del mesencefalo. La terapia rigenerativa dei DN potrebbe rappresentare un approccio promettente per il trattamento del PD. L'obiettivo di questo studio sperimentale è stato quello di valutare se le cellule staminali mesenchimali recentemente scoperte nelle cisti dentali infiammatorie periapicali umane (hPCy-MSC) 1 siano capaci di differenziarsi in DN funzionali al fine di utilizzarle come modello cellulare di studio. Materiali e metodi: Con approccio comparativo, HPCy-MSC e cellule staminali della polpa dentale (DPSC) sono state isolate ed esposte a condizioni neurogeniche (NC), su piastre di coltura (TCP) coatate con gel di poli-L-ornitina/laminina (gruppo rivestito) e TCP non coatate(gruppo non rivestito). Entrambi i gruppi sono stati anche coltivati in condizioni di coltura basale (BC),come controllo. In tutte le condizioni sperimentali: - Sono stati eseguiti saggi di biologia molecolare. -Sono state eseguite valutazioni al microscopio confocale. - Sono stati eseguiti test elettrofisiologici. -Sono stati anche studiati i livelli di lattato. - Sono stati prodotti dei Midbrain-like organoids in vitro. Risultati e conclusioni: I test di citometria a flusso ed immunofluorescenza hanno rivelato che le hPCy-MSC e le DPSC esprimono basalmente la β-III tubulina e la proteina gliale-fibrillare-acida (GFAP) 2;inoltre, sotto NC, le hPCy-MSC hanno mostrato una maggiore espressione genica in sensodopaminergico. I livelli di Neuromelanina (NM) erano significativamente più alti nel mezzo di coltura delle hPCy-MSC (rivestite e non rivestite) esposte a NC. Le hPCy-MSC sotto NC hanno altresì mostrato fluttuazioni oscillatorie spontanee in Vm, mai osservate nelle cellule indifferenziate. Infine, la diminuzione dei livelli di lattato nel mezzo delle hPCy-MSC sotto NC, ha confermato il differenziamento in senso neuronale. Clusterizzando, le cellule hanno poi formato sferoidi, ed in seguito strutture complesse similari ad una struttura complessa organoide. In conclusione, le HPCy-MSC si differenziano in DN funzionali, se esposte a NC specifiche. Gli organoidi simili al mesencefalo 3Dpotrebbero essere utilizzati per i trapianti autologhi, nonché per i modelli patologici per sviluppare terapie per il trattamento del morbo di Parkinson.

P55.

RECAPITULATING THYROID CANCER HISTOTYPES THROUGH ENGINEERING EMBRYONIC STEM CELLS

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Thyroid carcinoma (TC) is the most common malignancy of endocrine organs. The cell subpopulation in the lineage hierarchy that serves as cell of origin for the different TC histotypes is unknown. Human embryonic stem cells (hESCs) with appropriate in vitro stimulation undergo sequential differentiation into thyroid progenitor cells (TPCs-day22), which maturate into thyrocytes (day 30). Here, we created follicular cell-derived TCs of all the different histotypes based on specific genomic alterations delivered by CRISPR-Cas9 in hESC-derived TPCs. Specifically, TPCs harboring BRAFV600E or NRASQ61Rmutations generate papillary or follicular TC, respectively, whereas addiction of TP53R248Q generate undifferentiated TCs. Of note, TCs arise by engineering TPCs, whereas engineered thyrocytes do not generate TCs. The same mutations result in teratocarcinomas when delivered in early differentiating hESCs. TIMP-1/MMP-9/CD44 ternary complex, in cooperation with KISS1R, is involved in TC initiation and progression. Increasing iodide uptake, KISS1R targeting may represent a therapeutic adjuvant option for undifferentiated TCs.

P56.

SACUBITRIL/VALSARTAN IN AN EXPERIMENTAL MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Background. The majority of elderly patients with heart failure (HF) has a preserved ejection fraction (HFpEF). Despite high mortality and the growing social burden of HFpEF, the comprehension of its pathophysiology is incomplete, and treatment remains undefined. Based on the subgroup analysis from the PARAGON-HF trial, FDA extended the indication of sacubitril/valsartan to subjects with HFpEF, but uncertainty remains regarding its use in these patients. Aside from the possible clinical effect in HFpEF, the underlying mechanisms of the drug's effects in this syndrome are incompletely understood. Because chronologic aging contributes to deterioration of diastolic function, the objective of this work was to test the effects of sacubitril/valsartan in a model of age-related HFpEF.
Methods. 18-month-old female Fischer 344 rats were treated with oral administration of either sacubitril/valsartan (60 mg/kg/die, 1:1 ratio) or valsartan alone (30 mg/kg/die) for 12 weeks. Prior to treatments, animals were monitored by echocardiography. Tail-cuff method was used to measure blood pressure weekly. At the end of experiments, echocardiography and left ventricle catheterization were used to assess systolic and diastolic function. Masson's trichrome staining was used to detect fibrosis. Inflammation and oxidative stress measured by 3-nitrotyrosine, dihydroethidium and IL1 and IL6 expression. The components of RAAS pathways (ACE, ACE2, AT1, AT2 Mas1 receptors) were measured.
Results. In aging rats, diastolic function deteriorated without compromission of systolic parameters. Increased accumulation of fibrotic tissue in the aging hearts was evident together with inflammation and oxidative damage. None of the treatments efficiently changed diastolic dysfunction. Differently, both treatments led to a comparable reduction of hypertrophy and a decrease in systemic blood pressure. The treatments did not reduce inflammation and oxidative stress. Interestingly, a modulation of non-classic RAAS pathway was observed after combined sacubitril/valsartan treatment.
Conclusion. In age-related model of HEpEF, neither sacubitril/valsartan nor valsartan alone improve diastolic function although blood pressure and left ventricle thickness were reduced. The negative functional result may be related to the persistent chronic inflammation and oxidative stress and the accumulation of fibrotic tissue in the aged myocardium despite treatment. An effect on the non-classic RAAS pathways is noteworthy.

P57.

RENOPROTECTION BY DAPAGLIFLOZIN IN A NON-DIABETIC MODEL OF CARDIORENAL SYNDROME

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Background. Cardiorenal syndrome encompasses a spectrum of disorders involving heart and kidney dysfunction, and sharing common risk factors, such as hypertension and diabetes. Clinical studies have shown that patients with and without diabetes may benefit from using sodium-glucose cotransporter 2 inhibitors to reduce the risk of heart failure and ameliorate renal endpoints. Because the underlying mechanisms remain elusive, we investigated the effects of dapagliflozin on the progression of renal damage, using a model of non-diabetic cardiorenal disease.
Methods and Results. Dahl salt-sensitive rats were fed a high-salt diet for five weeks and then randomized to dapagliflozin or vehicle for the following six weeks. After treatment with dapagliflozin, renal function resulted ameliorated as shown by decrease of albuminuria and urine albumin-to-creatinine ratio. Functional benefit was accompanied by a decreased accumulation of extracellular matrix and a reduced number of sclerotic glomeruli. Dapagliflozin significantly reduced expression of inflammatory and endothelial activation markers such as NF-κB and e-selectin. Upregulation of pro-oxidant-releasing NADPH oxidases 2 and 4 as well as downregulation of antioxidant enzymes were also counteracted by drug treatment. Our findings also evidenced the modulation of both classic and non-classic renin-angiotensin-aldosterone system (RAAS), and effects of dapagliflozin on gene expression of ion channels/transporters involved in renal homeostasis.
Conclusion. Thus, in a non-diabetic model of cardiorenal syndrome, dapagliflozin provides renal protection by modulating inflammatory response, endothelial activation, fibrosis, oxidative stress, local RAAS and ion channels.

P58.

3D VASCULAR-SPHERES OF HUMAN MESENCHYMAL STEM CELLS DERIVED FROM ARTERIES.

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Mesenchymal Stem Cells (MSCs) are adult multipotent cells identified within several human tissues including arteries. Besides the fibroblast-like morphology, growth in adhesion and mesenchymal phenotype, MSCs have distinctive features as self-renewal, differentiation in multiple mesengenic lineages under appropriate stimuli and immunomodulatory properties. Another well-known MSC characteristic is the capacity to grow in a non-adherent condition as spheres. Here, we examined the attitude of MSC harvested from human vascular wall (hVW-MSCs) to form spheres when cultured in a 3D cell culture model; a phenotypic, molecular, morphological and metabolomic characterization of vascular-spheres was also provided.
hVW-MSCs were enzymatically isolated from human femoral arteries and in vitro expanded in a 2D culture model. To generate vascular-spheres, ultralow attachment and hanging drops techniques were employed. Immunophenotype and pluripotent genes were respectively analyzed by immunohistochemistry and Real Time PCR; morphology and ultrastructural organization through histology and electron microscopy; cell viability and proliferation with LIVE/DEATH and ki-67 proliferation marker, respectively; metabolomic profile using liquid chromatography - mass spectrometry.
In 2D cultures, hVW-MSC cells displayed an elongated morphology with mesenchymal antigens and multi-lineage differentiation potential. In 3D cultures, hVW-MSCs spontaneously formed floating vascular-spheres with CD44+, CD105low, CD90low profile expressing SOX2, OCT4 and NANOG stemness genes. In histology, vascular-spheres were organized in multiple layers of elongated cells enclosing round cells. Specifically, the
outer layer was composed of undifferentiated spindle cells with poor organelle content while the core was formed by rounded cells with a loosely arranged as observed in electron microscopy. Additional ultrastructural investigation of vascular-spheres highlighted the presence of a complex cell to cell communication system including nanotubular projections and multi-vesicular bodies enclosing exosomes. Furthermore, living cell were disposed in the outer layers, whereas dead ones in the core. Interestingly, hVW-MSCs spheres acquired a quiescent status as documented by low the percentage of ki-67+ cells and slowed metabolism for the increased accumulation of intermediate metabolites.
Taken together, these data proved that vascular-spheres of hVW-MSCs possess characteristics commonly found in stem niche as stemness, quiescence and reduced metabolic activity. This in vitro 3D model can be improved to deep the knowledge on hMSCs properties for future application in medical field.

P59.

GADD45B AS A POTENTIAL THERAPEUTIC TARGET IN ACUTE MYELOID LEUKEMIA

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Background: 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.

P60.

SETD8-MEDIATED P53 INACTIVATION AS AN EARLY PREREQUISITE OF INFLAMMATION-INDUCED COLORECTAL CANCER

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In many tumors, the oncosuppressor p53 is not mutated, but functionally inactivated. Restoring p53function remains an attractive therapeutic strategy especially for tumors that retain WT TP53. However, mechanisms of functional inactivation of p53 in these tumors remain poorly understood. Previously, we identified SETD8 as a crucial suppressor of p53 activity in Neuroblastoma. SETD8 is the sole enzyme that mono-methylates p53 on lysine 382 (p53K382me1), resulting in inhibition of its pro-apoptotic and growth arresting functions. We identified SETD8 inhibition as a therapeutic strategy to activate p53 in Neuroblastoma by decreasing p53K382me1. We found SETD8 overexpressed in several types of cancer with WT TP53 including, colorectal cancer (CRC). In 187 CRCs, we observed higher expression levels of SETD8 and p53K382me1 compared to healthy subjects and significantly longer disease-free survival in patients with low p53K382me1 levels. p53K382me1 is an independent factor associated with prognosis even in stage 4 CRCs. Notably, p53K382me1 is highly expressed in cancer stem cells and macrophages in tissues derived from CRC patients. Patients with Crohn's disease or ulcerative colitis who subsequently developed CRC, at IBD diagnosis expressed high levels of p53K382me1 in the immune compartment, suggesting that p53K382me1 may be an early prerequisite of tumorigenesis in inflammation-induced CRCs.

P61.

HYDROGEL SELECTION FORFIBROBLAST-LIKE SYNOVIOCYTESENCAPSULATIONAND 3D CULTURINGFOR THE DEVELOPMENT OF A SYNOVIA-ON-CHIP

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Rheumatoid arthritis (RA) is one of the most prevalent chronic inflammatory diseases. It is considered a complex syndrome with autoimmune and inflammatory features. RA is characterized by a progressive symmetric inflammation of affected joints, resulting in cartilage destruction, bone erosion, and disability. Treatments for RA, both conventional and biologics are available, but only a small proportion of patients benefit from them (40%). The aim of precision medicine is to stop this trial-and-error approach and find a way to stratify patients so that each single patient would receive a tailored treatment, to maximize its efficacy and minimize its side effects. To this aim, it would be beneficial to have a personalized and physiologically relevant disease model, to test all the available drugs on the market at once. In this frame, the specific aim of this project is to develop a Synovia-on-Chip (SoC),using patient-derived synovial biopsies. Microinvasive eco-guided synovial biopsy is indeed an easy and safe procedure, that allows to retrieve synovial fibroblasts from the inflamed joints of each patient. Synovial fibroblasts were disaggregated from the tissue biopsy with the employ of collagenase and initially cultured in two-dimensional (2D)culture from passage I to passage VII, in order to describe if and how culturing would affect the phenotype of FLS over time. The expression of FLS-markers was evaluated by flow cytometry using Live/Dead in combination with human monoclonal antibodies anti-CD45, anti-CD34, anti-CD90, and anti-CD55. Results showed that cells from passage III to passage VI were in the optimal differentiation status. FLS were embedded in two different biopolymers (chitosan, ch, and gelatin methacrylate, GelMa) and observed at different time points through fluorescence microscopy to monitor their viability and morphology. Results showed that in both hydrogels, cells maintain overall a good viability. Despite this, we noticed that in the ch-based hydrogel, FLS appeared rounded and isolated, incapable of stretching to reach their typical morphology. Instead, the embedment in GelMa showed promising results: cells maintained a full viability and were characterized by a great proliferation rate. Furthermore, in this formulation, cells were finally able to elongate, and establish a net among cells. Considering the results, we concluded that GelMa is a good candidate for FLS embedment and could be applied for SoC development.

P62.

SEM/EDS EVALUATION OF THE REMINERALIZATION EFFICACY OF FOUR REMINERALIZING AGENTS ON ARTIFICIAL ENAMEL LESIONS

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In recent years, the focus in caries research has shifted to the development of methodologies for the non-invasive management of early caries lesions through remineralization to preserve tooth structure and prevent the early enamel lesions progression. Hence, the aim of the present study was to analyze and compare the remineralizing effectiveness of four different commercially available agents on artificial human enamel lesions after seven days of treatment, by means of Scanning Electron Microscopy (SEM) combined with Energy Dispersive Spectroscopy (EDS) techniques. Thirty-six extracted third molars were collected and randomly assigned to six groups (n=6), five of which were suspended in demineralizing solution for 72 h to create enamel artificial lesions, and one serving as control: G1, treated with a mousse of casein phosphopeptide and amorphous calcium–phosphate (CPP-ACP); G2, treated with a gel containing nano-hydroxyapatite (N-HA); G3, treated with a 5% Sodium Fluoride (SF) varnish; G4, treated with a toothpaste containing ACP functionalized with fluoride and carbonate-coated with citrate (F-ACP); G5, not-treated artificial enamel lesions; G6, not demineralized and not treated sound enamel. G1–G4 were subjected to pH cycling over a period of seven days. Analyses of the specimens' enamel surfaces morphology were performed by SEM and EDS. Data were statistically analyzed for multiple group comparison by one-way ANOVA/Tukey's test ($p < 0.05$). The results show that the Ca/P ratio of the G5 (2.00 ± 0.07) was statistically different ($p < 0.05$) from G1 (1.73 ± 0.05), G2 (1.76 ± 0.01), G3 (1.88 ± 0.06) and G6 (1.74 ± 0.04), while there were no differences ($p > 0.05$) between G1, G2 and G6 and between G4 (2.01 ± 0.06) and G5. The initial enamel demineralization surface may be treated with topical use of remineralizing agents, achieving an almost complete remineralization of the surface and a reorganization of the prismatic structure of the enamel. In all groups tested, after seven days of remineralizing agents' application, complete remineralization was not obtained, but rather a reorganization of the enamel structure from both quantitative and qualitative points of view.

P63.

DRUG COMBINATION THERAPY FOR INHIBITING HSV-1 INFECTION

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Herpes simplex virus (HSV) infection causes significant disease globally. In immunocompromised people, such as those with HIV infection, HSV-1 can lead to severe symptoms, such as keratitis (eye infection) or encephalitis (brain infection). Antiviral drugs for the treatment of HSV infections have been developed over the past 40 years. However, most drug resistant HSV isolates have been discovered in laboratories and clinics. The identification of novel strategies for the development of new antiherpetic molecules with different mechanisms of action is challenging, particularly for the management of severe and otherwise hard to treat HSV infections. In this scenario, we analyzed the presence of over-represented transcription factor binding sites (TFBSs) in the entire HSV-1 genome. Yin Yang 1 (YY1) is an important mediator of different processes, such as cell growth, differentiation, tumor development and apoptosis. It acts in a dual mode depending on the recruited cofactors, activating or inhibiting gene expression. In addition to a direct regulation of gene transcription, it is well known its involvement in epigenetic modulation. Nevertheless, its role is not yet clear and it can represent an innovative molecular target in HSV-1 infections. In order to evaluate the role of YY1 in the HSV-1 regulation, we performed a bioinformatic analysis and YY1 was statistically the most represented and enriched protein. To understand YY1 role in the regulation of HSV-1 transcription, epithelial cells were pretreated with YY1 inhibitor NPI-0052 and, subsequently, infected with HSV-1. Our data showed that NPI-0052 was able to inhibit viral infectivity in a dose-dependent manner, indicating that it could interact with the cell reducing the HSV-1 entry. Although YY1 is a direct regulator of transcription, it is found in many chromatin regulation complexes. Among the most characterized and known "partners" there is the family of Histone deacetylases (HDACs). Then the inhibitory effect of NPI-0052 in combination with different HDACs and EZH2 inhibitors was evaluated against HSV-1. The infection was monitored by plaque-assay and we noted that the viral infection was totally inhibited, indicating an interaction between YY1 and transcriptional repressors, such as HDACs and EZH2. Finally, a chromatin immunoprecipitation (ChIP) was performed with the antibody against YY1, followed by qPCR using viral gene primers. The results showed that YY1 recognizes the viral gene promoters and, following treatment with NPI-0052 and epigenetic modulators, this recognition was totally removed. The multidrug approach between the YY1 inhibitor NPI-0052 and several epigenetic modulators could represent a new and effective therapeutic strategy in the prevention and recovery of HSV infection, especially in severe cases.

P64.

PREDICTING DIFFERENT TUMOR TYPES FROM MOLECULAR DATA USING FEATURE EXTRACTION AND MACHINE LEARNING APPROACHES

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Introduction: Cancer is a complex disease, with patterns of genomic alterations showing intra-cancer heterogeneity and cross-cancer similarity. 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 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, the following two strategies were considered: -set a percentage cut-off threshold to remove from the analysis less represented cancer types, -divide cancer types into different groups, and training a specific XGBoost model for each of them.
Results: Using different cut-off thresholds, the XGBoost classifier achieved the best performance (accuracy of 77%, AUC score of 97%) on a dataset containing only 10 out of 32 tumor types.
By removing poorly represented cancer types and dividing tumors into 3 different groups (endocrine-related carcinomas, other carcinomas and other cancers), a total of 18 different tumor types were analyzed and the 3 analysis models achieved 78%, 71% and 86% accuracy respectively, with AUC scores above 96%. 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 81% on the holdout data and a 94% AUC score.
Conclusion: A new accurate ML model discriminating among different cancer types based on somatic alterations was developed. Although further analyses to confirm the performance of this promising model as well as extract information of biological relevance are required, this approach could have potential clinical application in terms of cancer diagnosis improvement.

P65.

LOSS OF ATP2C1 FUNCTION PROMOTES ENDOCYTOSIS AND DEGRADATION OF NOTCH1, IMPLICATIONS FOR HAILEY-HAILEY- DISEASE

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Hailey-Hailey Disease (HHD) is a rare autosomal dominantly inherited disorder caused by mutations in the ATP2C1 gene that encodes an adenosine triphosphate (ATP)-powered calcium channel pump. HHD is characterized by impaired epidermal cell-to-cell adhesion and defective keratinocyte growth/differentiation. The mechanism by which mutant ATP2C1 causes HHD is unknown and current treatments for affected individuals not address the underlying defects and are ineffective. Notch signaling is a direct determinant of keratinocyte growth and differentiation. We found that loss ofATP2C1 leads to impaired Notch1 signaling, thus deregulation of the Notch signaling response is therefore likely to contribute to HHD manifestation. NOTCH1 is a transmembrane receptor and upon ligand binding, NOTCH intracellular domain (NICD) translocates to the nucleus activating its target genes. In the context of HHD we found that loss of ATP2C1 function promotes upregulation of the active NOTCH1 protein, (NICD-Val1744). Here, deeply exploring this aspect, we observed thatNOTCH1 activation is not associated with transcriptional enhancement of its targets. Moreover, in agreement with these results, we found a cytoplasmic localization of NICD-Val1744. We have also observed that ATP2C1-loss is associated with degradation of NICD-Val1744 through the lysosomal/proteasome pathway. These results show that ATP2C1-loss could promotes a mechanism by which NOTCH1 is endocytosed and degraded by the cell membrane. The deregulation of this phenomenon, finely regulated in physiological conditions, could in HHD lead to the deregulation ofNOTCH1 with alteration of skin homeostasis and disease manifestation.