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"Organoids as models of human diseases"

ABSTRACT BOOK

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ORAL COMMUNICATION

POSTER

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CO01. A multifunction platform simulating human intestinal physiology to study the effects of ingested micro- and nano-particles

Author(s): Giulia Antonello[a,b,c], Marianna Esposito[b], Giulia Orlandin[a], Francesco Barbero[b], Ivana Fenoglio[b], Chiara Riganti[c], Enrico Bergamaschi[a]

Institution(s): a.Department of Public Health and Pediatrics, Piazza Polonia, 94, University of Turin, 10126 Torino, Italy, b.Department of Chemistry, University of Turin, Via Pietro Giuria 7, 10125 Torino, Italy, c. Department of Oncology, University of Turin, Via Santena 5 bis, 10126 Torino, Italy.

Abstract: Human exposure to micro- and nano-materials (MNMs) is rising because of their widespread production and use. Despite ingestion is considered one of most common exposure routes, it still difficult to study the effects of MNMs in vitro because advanced models that reproduce the complexity of human gut are lacking. Here, we propose a multifunction in vitro platform that fills this gap, obtained by combining advanced co-culture models, dynamic conditions and in vitro Human Simulated Digestion System (SHDS) to better assess the consequences of MNMs ingestion. By co-culturing enterocyte-like and goblet cells with lymphocytes B we obtained and validated a gut model with most relevant features of a physiological intestinal barrier (presence of tight junctions, mucus, and microfold cells). Applying a flow rate in the culture chamber, we mimicked the physiological pressure gradient that drives the passage of solutes, molecules and particles through the intestinal barrier. In this way, we could monitor the impact of SHDS on MNMs degradation, aggregation/agglomeration, bio-corona formation and their effects on cell viability, barrier integrity, inflammation, and MNMs passage across the barrier, by exposing our reconstituted model to MNMs, subjected or not to SHDS1. Our multifunctional platform is a useful tool to study the biological effects of ingested particles, tracking the impact of digestion and the changes in key physiological features of the intestinal barrier, potentially representing a future alternative to in vivo models.

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CO02. Hedgehog-GLI and NOTCH signalling pathways induce chemotherapeutic resistance and mesenchymal phenotype in Colorectal cancer organoids

Author(s): Anna Citarella[a], Giuseppina Catanzaro[a], Zein Mersini Besharat[a], Sofia Trocchianesi[b], Federica Barbagallo[c], Giorgio Gosti[d], Marco Leonetti[d], Annamaria di Fiore[b], Lucia Coppola[a], Tanja Milena Autillio[a], Elena Splendiani[b], Alessandra Vacca[a], Mary Anna Venneri[a], Enrico De Smaele[a], Elisabetta Ferretti[a], Agnese Po[b]

Institution(s): a Department of Experimental Medicine, “Sapienza” University of Rome, Rome, Italy. b Department of Molecular Medicine, “Sapienza” University of Rome, Rome, Italy c Faculty of Medicine and Surgery, Kore University of Enna, Enna, Italy d Soft and Living Matter Laboratory, Institute of Nanotechnology, Consiglio Nazionale delle Ricerche, Rome, Italy.

Abstract: Colorectal cancer (CRC) is a leading cause of mortality and morbidity. CRC patients frequently present chemoresistance, achieved by the modulation of cell survival signaling pathways. Hedgehog-GLI (HH-GLI) and NOTCH pathways are involved in stemness features of normal colonic mucosa, and their mis-activation in CRC is associated with poor prognosis and epithelial to mesenchymal transition (EMT) process. In the present study we evaluated the role of HH-GLI and NOTCH pathways as regulatory mechanisms responsible for the chemotherapy resistance and invasive phenotype in CRC organoids. CRC cell lines, HCT116 (KRAS mutant) and HT29 (BRAF mutant), were cultured as monolayer and organoids (oHCT116 and oHT29) and were treated with the chemotherapeutic agent 5- Fluorouracil (5-FU) alone or in combination with HH-GLI and NOTCH pathways inhibitors, GANT61 and DAPT respectively, and arsenic trioxide (ATO) to inhibit both pathways. We reported that in both cell lines, 5-FU treatment led to the mis-activation of HH-GLI and NOTCH pathways, that cooperated, resulting in the escape from apoptosis in 2D cultured cells and in the promotion of the EMT phenotype in 3D organoids. Indeed, 5-FU treated CRC organoids, oHCT116 and oHT29, showed up-regulation of Vimentin, ABCG2, CD133a and c- MET. Interestingly, we reported the physical features of invasiveness by studying single-cell trajectories in GFP-labelled oHCT116 treated with 5-FU, through 3D live imaging. Our data highlight the role of both HH-GLI and NOTCH pathways in the regulation of CRC mesenchymal phenotype. Moreover, our results describe how the use of GLI1 and NOTCH inhibitors could be useful in overcoming CRC chemoresistance.

CO03. Soundwave-driven vascularized 3D models of cancers: a reliable tool for drug screening

Author(s): Joanna Kopecka[a,b], Nicola Di Marzio[c,d], Preeta Ananthanarayanan[a], Anne Geraldine Guex[c], Mauro Alini[c] ,Chiara Riganti[a,b], Tiziano Serra[c]

Institution(s): [a]Department of Oncology, University of Torino, 10126, Torino, Italy, [b]Inter-departmental Centre "G. Scansetti" for Studies on Asbestos and Other Toxic Particulates, University of Torino, 10126, Torino, Italy, [c]AO Research Institute Davos, 7270 Davos, Switzerland, [d]Department of Health Sciences, Universita Del Piemonte Orientale (UPO), Novara, Italy,

Abstract: The tumor mass consists of a heterogeneous population of cancer, stromal, immune cells that together with vascular system, secreted factors and extracellular matrix proteins build so called tumor microenvironment (TME). TME have important role in cancer progression, and influences drug response. Therefore, suitable *in vitro* models recreating tumor and its microenvironment is a mandatory step in each drug screening study. Here we present a new and highly innovative soundwave-driven vascularized 3D model [1] of malignant pleural mesothelioma, chosen as prototype of highly drug resistant tumor for which organoids are not available yet. Our model, created using a soundwave-driven and defined spatial cell patterning in fibrin hydrogel, is composed from tumor heterotypic spheroids, consisting of fibroblasts and primary malignant pleural mesothelioma cells, and is surrounded by a vascular ring of competent microvessels. Thanks to the mild hydrodynamic forces used to shape the cells, this models are highly reproducible and can be easily scaled-up for high-throughput screening. The growth of both heterotypic tumor spheroids and microcapillary network can be easily monitored in real time. Thismodel is suitable to perform drug efficacy screening, using both tumor-targeting and anti-angiogenic agents, and transcriptome analysis to set the bases for mechanistic studies. We propose the sound patterning as a fast, scalable and cell-friendly approach, able to spatially recreate TME *in vitro* and feasible for primary tumors that have not well-established organoids protocols. Our platform is suitable to monitor drug response and test personalized treatments.

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CO04. Pharmacological targeting of the novel β-catenin chromatin-associated kinase p38α in colorectal cancer stem cell tumorspheres and organoids

Author(s): Martina Lepore Signorile[a], Valentina Grossi[a], Giovanna Forte[a], Candida Fasano[a], Katia De Marco[a], Paola Sanese[a], Vittoria Disciglio[a], Cristiano Simone[a]

Institution(s): a Medical Genetics, National Institute for Gastroenterology, IRCCS 'S. de Bellis', Castellana Grotte (Ba), 70013, Italy b Medical Genetics, Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Bari, 70124, Italy

Abstract: Locally advanced colorectal cancer (CRC) has a 5-year survival rate of approximately 60%. This is mainly due to drug resistance, recurrence, and subsequent metastatic dissemination, which are sustained by the cancer stem cell (CSC) population. The main driver of the CSC gene expression program is Wnt signaling, and previous reports indicate that Wnt3a can activate p38 MAPK. Besides, p38 was shown to feed into the canonical Wnt/β-catenin pathway. We previously showed that p38α is required to maintain CRC metabolism and survival, as its inhibition leads to activation of autophagic cell death in vitro and in vivo. Here, we show that stage III CRC patients with high p38α levels have reduced disease-free and progression-free survival and that stage III patient-derived CRC-SCs have higher levels of activated p38α than normal colonocytes and are "addicted" to p38α activity. Importantly, we found that p38α is the β- catenin chromatin-associated kinase required for the regulation of a signaling platform involved in tumor proliferation, metastatic dissemination, and chemoresistance. Our results also showed that p38α kinase inhibitor Ralimetinib decreases patient-derived CRC-SCs proliferation, migration ability and, more importantly, enhances the synthetic lethality effect of the MEK/ERK inhibitor Trametinib and the sensitization of chemotherapeutic drugs commonly used in CRC, such as 5- FU, Cisplatin and Irinotecan, promoting a reduction in cell proliferation and a significant increase in cell death. Taken together, these results suggest that p38α may be targeted in CSCs to devise new personalized CRC treatment strategies.The functional relationship between p38α and β- catenin was characterized in vitro, in cellulo by exogenous and endogenous analysis (CRC cell lines, patient-derived CRC-SC tumorspheres and APCMin/+ mice intestinal organoids) and in vivo models (C57B/l6-APC+/-Min).

CO05. Generation of blood vessel organoids: a powerful model to investigate endothelial dysfunction during insulin resistance

Author(s): Elisa Martino[a], Camilla Anastasio[a], Antonino Colloca[a], Nunzia D'Onofrio[a] and Maria Luisa Balestrieri[a]

Institution(s): [a]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", via L. De Crecchio 7, 80138 Naples, Italy.

Abstract: Blood vessels are fundamental to human life and play critical roles in many chronic diseases, including diabetes and insulin resistance (IR). Endothelial cells (EC) are critical for cardiovascular disease therapy, vascular regeneration, personalized drug development, and tissue engineering. Human pluripotent stem cells (hPSCs) afford us with an unprecedented opportunity to produce unlimited EC and to generate blood vessel organoids. To date, the concept of endothelial IR has not been clinically established because only a small number of clinical studies have shown the actual response to insulin in EC of patients with metabolic disorders. To this end, the effects of palmitic acid (PA) on oxidative homeostasis has been firstly evaluated in human TeloHAEC 2D model. Results indicated that 48 h of treatment with PA (0.5 mM) impaired cell viability, induced loss of insulin signaling, and imbalanced the intracellular and mitochondrial oxidative status ($p<0.001$). Moreover, PA treatment promoted pyroptosis by the NLRP3/caspase-1 axis ($p<0.001$), inducedautophagic mechanism ($p<0.01$) and caused negative modulation of the mitochondrial guardian sirtuin SIRT3 ($p<0.001$). SIRT3 overexpression by mimic ($SIRT3^+$) suppressed the PA-induced oxidative damage ($p<0.01$), as well as autophagy ($p<0.01$), inflammation, and pyroptosis ($p<0.01$). Overall, results support the central role of SIRT3 in endothelial redox impairment occurring under IR stress. Future experiments will require the development of self-organizing 3D human blood vessel organoids from hPSCs in order to faithfully recapitulate the structure and function of human blood vessels, to identify the epigenetic regulators of IR and to accelerate metabolic dysfunction research.

CO06. 3D-model versus 2D-model of Glioblastoma multiforme: two different model to overcome the low drug delivery through blood-brain barrier

Author(s): Iris Chiara Salaroglio[a], Sabrina Digiovanni[a], Joanna Kopecka[a], Pierre-Olivier Couraud[b], Chiara Riganti[a].

Institution(s): a. Dipartimento di Oncologia, Università degli Studi di Torino, Torino.
b. Institut Cochin, Université de Paris, Inserm U1016/CNRS UMR8104, Paris, France.

Abstract: Glioblastoma multiforme (GBM) is a highly aggressive tumour, difficult to treat with chemotherapy due to the blood-brain barrier (BBB), rich of tight junctions (TJs) and ATP- binding cassette transporters like P-glycoprotein (Pgp), which effluxes drugs backward. BBB is often disrupted within GBM [1], but the mechanisms are unknown.
To address this issue, we set up organotypic cultures of human competent BBB and patient-derived GBM cells. From each of them, we generated organotypic cultures containing only GBM stem cells, grown as 3D-neurospheres (NS), and differentiated GBM cells, which grow as 2D-adherent cells (AC) [2]. By exploiting these organotypic cultures, we investigated if the GBM stemness/differentiation degree impact on drug delivery across BBB. The presence of GBM cells co-cultured with BBB increased the permeability to doxorubicin and dextran-70, compared to BBB alone, transcriptionally decreasing the expression of Pgp and TJ proteins. The increase in drug delivery and BBB permeability was higher with AC than with NS, and with AC-conditioned medium than with NS-conditioned medium. The secretome analysis identified IL-6 as significantly higher in AC than in NS-medium. AC-medium containing anti-IL-6 neutralizing antibody reduced the BBB permeability to NS levels, while NS-medium enriched with IL-6 increased BBB permeability to AC levels. Mechanistically, AC- or NS-produced IL-6 differentially modulated STAT3 signalling in BBB cells, explaining the effects on permeability, Pgp and TJs. Our study pointed out the need of using suitable organotypic models that recapitulate the biological complexity of real tumors and their microenvironment. In particular, thanks to the GBM/BBB models produced, we dissected how the degree of stemness/differentiation of GBM may affect the drug delivery and the consequent response to chemotherapy.

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CO07. Regulatory role of miR-449a as a potential biomarker in Head and Neck Carcinoma

Author(s): Federica Melisi[a,b*], Alessia Maria Cossu[a,b*], Piera Grisolia[a,b], Lucia Pasquale[b], Marco Bocchetti[b], Teresa Maria Noviello[c,d], Michele Ceccarelli[c,d], Gabriella Misso[a], Michele Caraglia[a,b], Marianna Scrima[b]

Institution(s): [a]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples-Italy, [b]Biogem Scarl, Laboratory of Molecular and Precision Oncology, 83031 Ariano Irpino-Italy, [c]Department of Electrical Engineering and Information Technology, University of Naples "Federico II", Napoli,[d] Biogem Scarl, Laboratory of Bioinformatics, 83031 Ariano Irpino-Italy

Abstract: Laryngeal cancers represent one-third of all head and neck cancers (HNC) and maybe a significant source of morbidity and mortality. Diagnosis often occurs in a late phase, resulting in delayed treatment and worse prognosis. Therefore, successful clinical management is strictly linked to the identification of reliable diagnostic and prognostic biomarkers [1]. MicroRNAs could represent potential candidates as early noninvasive biomarkers. Our study began with the characterization of miR-449alevels in laryngeal cancer patients that showed a significant decrease of miR-449a in patients with nodal metastasis [2]. In order to identify the biological role of miR-449a, we evaluated its relative expression levels in different HNC cell lines by RTqPCR analysis. The analysis revealed a different expression of miR-449a in HNO-210 and FaDu tumor cell lines. In order to study miR-449a biological activity and its role in biomolecular processes, HNO-210 and FaDu tumor cell lines were stably transduced with miR-449a. In HNO-210 stable cell line, we performed NGS WholeTranscriptome Rna-seq analysis to understand molecular pathways deregulated by miR449aoverexpression. Sequencing results showed a downregulation of IL6R gene in miR-449a overexpressing cell line and a modulation of Jak-STAT pathway. We confirmed miR-449a directly binds to the 3' UTR of IL6R gene. Results demonstrate that down regulation of the IL6-R in miR-449a HNO-210 cell model reduces the phosphorylation levels of downstream signaling, blocking JAK/STAT pathways that could induce tumor transformation.We provide evidence of the role of miR-449a regulation of IL6/JAK/STAT on laryngeal cancer *in vitro* models.

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CO08. A combined approach based on kidney cell lines and kidney organoids led to the Identification of a novel role of OCRL in Lipid Droplets dynamics unveiling the molecular mechanisms underneath thedecline of kidney function in Lowe Syndrome

Author(s): Francesca Cervellini[a], Marialuisa Testa[a], Lidia Caserta[a], Michele Santoro[a], Maria Antonietta De Matteis[a,b] and Leopoldo Staiano[a,c].

Institution(s): [a]Telethon Institute of Genetics and Medicine, Pozzuoli, Naples, Italy, [b] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy, [c] Institute for Genetic and Biomedical Research, National Research Council (CNR), Milan, Italy.

Abstract: Fatty Acids (FAs) are transported from Lipid Droplets (LDs) toperoxisomes and mitochondria where they are used as a source of energy. Kidney proximal tubule epithelial cells mostly rely on FAs metabolism for energy production and its impairment results in severe cell damage and cell death. Phosphatidylinositol 4,5 bisphosphate (PI4,5P2) favors the contacts between LDs and peroxisomes allowing FAs metabolism and transport to mitochondria. PI4,5P2 level is increased in Lowe Syndrome (LS), a rare genetic disease caused by mutations in OCRL, a PI4,5P2 5-phosphatase characterized by congenital cataracts, intellectual disabilities, and Renal Fanconi Syndrome. The decline of kidney function towards chronic kidney disease (CKD) has been observed in several LS patients, although its pathogenesis is understudied. To discover new molecular mechanisms of proximal tubule dysfunction and CKD in LS, we investigated the role of OCRL and PI4,5P2 in the regulation of LDs-peroxisomes-mitochondria interplay and its effect on FAs metabolism. In kidney proximal tubule cells depleted of OCRL, PI4,5P2 accumulates on LDs and peroxisomes reducing the transport of fueling lipids to mitochondria, that display lower mitochondrial membrane potential, increased ROS levels and structural abnormalities. Cells and kidney organoids lacking OCRL have an increased number of PI4,5P2-rich LDs because of the combined impairment of LDs contact to peroxisomes and?. Finally, kidney organoids lacking OCRL showincreased deposition of extracellular matrix and thus increased fibrosis, that is a hallmark of CKD. In summary, this study is paving the way to a wider understanding of kidney dysfunction in LS and aims at the discovery of new fundamental cell biology mechanisms controlled by PI4,5P2 and OCRL.

CO09. Identification of prognostic gene markers using transcriptome analysis in hypoxic mesothelioma

Author(s): Madhura Tathode[a], Caterina Costa[b], Marianna Scrima[c], Alessia Maria Cossu[a,c], Silvia Zappavigna[a], Pierpaolo Correale[d], Antonio Giordano[e,f], Luciano Mutti[g], Francesca Pentimalli[h], Michele Caraglia[a,c]

Institution (s): a. Department of Precision Medicine, University of Campania "L. Vanvitelli", Naples, Italy; b. Cell Biology and Biotherapy Unit, Istituto Nazionale Tumori, IRCCS, Fondazione G Pascale, Naples, Italy; c. Biogem Scarl, Ariano Irpino, Italy; d. Medical Oncology Unit, Grand Metropolitan Hospital "Bianchi- Melacrino-Morelli", Reggio Calabria, Italy; e. Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, PA, USA; f. Department of Medical Biotechnologies, University of Siena, Siena, Italy; g. Department of Biotechnological and Applied Clinical Sciences (DISCAB), University of L'Aquila, Italy; h. Department of Medicine and Surgery, LUM University, Casamassima (BA)

Abstract: Malignant Mesothelioma (MM) is a type of cancer arising from the mesothelium, a thin layer of cells, lining internal organs. Primarily caused by asbestos exposure, over 30,000 people are diagnosed with MM annually. Therapeutic measures, which include chemo- and radiotherapy, and surgery in some cases, poorly increase life expectancy and there is no permanent cure for this disease. Therefore, early detection and prognostic prediction are important to improve the survival in MM patients. Pleural MM induces respiratory distress leading to hypoxia. Hypoxia, in turn, leads to accumulation of adenosine which favors tumor progression by suppressing T cell mediated immune response [1]. Targeting adenosine receptors recently emerged as a promising anti-tumoral strategy. To mimic active hypoxia, mesothelioma cell line REN was treated with NECA [an adenosine receptor agonist] for 2 hours which effectively induced intracellular CREB phosphorylation [2]. Then, by whole transcriptomics analysis, we studied expression pattern of NECA-treated cells compared to control. We found a total of 199 differentially expressed genes (DEGs) and non-coding RNAs after NECA treatment. Survival related DEGs and lncRNAs associated with mesothelioma patient prognosis were identified in silico using GEPIA2 and lncSEA, respectively. Further, protein coding genes and lncRNAs were shortlisted based on significant p-value (<0.05) in differential expression and GEPIA2 overall survival (OS) analysis for experimental validation. In KEGG Pathway & Gene Ontology analysis we have found significant regulation of oxygen transport and ribosome complex formation confirming a role of adenosine in the hypoxia process. RT-PCR analysis confirmed increased expression of CNIH2 (Cornichon Family AMPA Receptor Auxiliary Protein 2) and decreased expression of GLN1 (G protein nucleolar 1) in NECA treated mesothelioma cells. These protein coding genes are associated to significant changes in OS of MM patients suggesting a role for these proteins as both prognostic and therapeutic targets.

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POSTER

P01. Novel strategy in nanoparticle-based miRNA delivery to overcome chemoresistance in glioblastoma via HMGA2

Author(s): Marianna Abate*[a], Marco De Martino*[a], Francesco Esposito[d], Rossella Di Paola[a], Angelica Di Matteo[a], Lorena Scotti[c], Virginia Campani[c], Giuseppe De Rosa[c], Paolo Chieffi[e], Michele Caraglia[a,b], Silvia Zappavigna[a].
* Co-First Authors

Institution(s): [a] Department of Precision Medicine University of Campania "Luigi Vanvitelli", Naples, Italy. [b] BIOGEM scarl Institute of Genetic Research, Ariano Irpino, Italy. [c] Department of Pharmacy, University of Naples "Federico II", Naples, Italy. [d] Department of Molecular Medicine and Medical Biotechnology, University of Naples 'Federico II', Naples, Italy [e] Department of Psicology, University of Campania "Luigi Vanvitelli", Naples, Italy.

Abstract: Glioblastoma (GBM) is the most malignant type of Glioma. It is currently treated with radiotherapy in combination with temozolomide (TMZ), but this neoplasm remains with a fatal outcome. Nevertheless, cytotoxic activity of TMZ is strongly influenced by DNA damage repair mechanisms. HMGA plays a critical role for maintaining stem-cell state and chemoresistance in GBM. *In silico* analysis found High Mobility Group A (HMGA) proteins among targets of miR-603. To confirm bioinformatics data, HMGA2 binding sequences for miR-603 and their corresponding mutants were inserted into the multiple cloning site downstream the firefly luciferase gene. MiR-603 induced adecrease of firefly luciferase expression in cells transfected withthe construct carrying the HMGA2 binding sequences but not with their corresponding mutants. Furthermore, the targeting of HMGA2 by miR-603 was confirmed by western blot and RT-PCR in T98G and U87MG, suggesting an inverse correlation between the expression of HMGA2 and miR-603. HMGA2 is involved in cell proliferation, migration and invasion, we demonstrated that cell transfection with miR-603 significantly reduced these mechanisms. These results were confirmed when our cellmodels were transfected with a siRNA against HMGA2, while overexpression of HMGA2 antagonized miR-603-induced effects. In order to evaluate the ability of miR-603 delivery to overcome GBM resistance to TMZ, we have carried out the experiments of combination between SANPs encapsulating miR-603 and TMZ on GBM cells. miR-603, encapsulated in different SANP formulations, was delivered into GBM cells, which were subsequently treated with TMZ. When SANP-miR603 and TMZ were used in combination, we found a significant growth inhibition in T98G cells and at a lesser extent in U87MG. In vitro experiments on GBM organoid models to evaluate the ability of miR-603 to restore TMZ sensitivity targeting GBM stem cells are ongoing. Therefore, miR-603 could be a powerful tool to overcome chemoresistance in the treatment of GBM.

P02. Environmental Flame Retardant Pollutants And Thyroid Disease: Experimental Model For Evaluation Of Growth Signals And Dna Base Excision Repair.

Author(s): Gitana Maria Aceto [a], Maria Ersilia Talone [a], Lisa Marinelli [b], Maria Carmela Di Marcantonio [c], Anna Cichella [d], Antonio Di Stefano [b], Roberto Cotellese [a,e].

Institution(s): [a] Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Via dei Vestini, 66100 Chieti, Italy, [b] Department of Pharmacy, University "G. D'Annunzio", Via dei Vestini 31, 66100, Chieti, Italy, [c]Department of Innovative Technologies in Medicine & Dentistry, University "G. 'Annunzio" Chieti-Pescara, 66100 Chieti, Italy, [d] Department of General Surgery, Private Hospital "Villa Serena", Città Sant'Angelo, 65013 Pescara, Italy, [e] Villa Serena Foundation for Research, Città Sant'Angelo, 65013 Pescara, Italy.

Abstract: In this study, the molecular impact of the flame retardant pollutant triphenyl phosphate (TPhP) was evaluated in vitro using the Nthy-ori 3-1 model derived from normal human thyroid follicular epithelium. Particular attention was given to the expression of signals involved in thyroid pathophysiology, including thyroperoxidase enzyme (TPO), basic excision repair system (BER), EGFR and ErbB2. The effects were assessed by the following methods: MTS for cell viability, Wound Healing for cell motility, ROS-Glow for H_2O_2 production, qReal-Time-PCR for gene expression, Western Blot for protein expression, and HPLC analysis to assess intracellular and extracellular concentrations of the DPHP metabolite. Exposure of Nthy-ori 3-1 to 2μM and 10μM triphenyl phosphate (TPhP) and its metabolite diphenyl phosphate (DPhP) after 24 hours resulted in increased cell viability and H_2O_2 production. In gene expression analysis, both treatments induced a clear increase in the expression of genes involved in the BER system and of TPO, EGFR and ErbB2. DPhP increased the expression of BER, MUTYH and APE1/Ref proteins, but also that related to ErbB2, confirming a dysregulation of cellular physiology. This study demonstrated, for the first time, that following exposure of the tissue microenvironment to the metabolite DPhP, thyroid cells undergo upregulation of growth and oxidative DNA damage repair signals, due to a plausible increase in cellular stress, found not only with increased H_2O_2production, but also with increased mitochondrial activity and cell growth. Further clinical and experimental observations will be needed to obtain a complete picture of the effects of these pollutants on thyroid gland function.

P03. Protective Effect of Resveratrol against Hypoxia-Induced Neural Oxidative Stress

Author(s): Amogh Auti[a], Nicola Alessio[b] , Andrea Ballini[a] , Mario Dioguardi[c] , Stefania Cantore[d], Salvatore Scacco[e], Antonio Vitiello[f], Lucio Quagliuolo[a], Barbara Rinaldi[b], Luigi Santacroce[g], Marina Di Domenico[a,h] and Mariarosaria Boccellino[a]

Institution(s): a. Department of Precision Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy; b. Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy; c. Department of Clinical and Experimental Medicine, University of Foggia, 71122 Foggia, Italy; d. Independent Researcher, 70129 Bari, Italy; e. Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari ALDO MORO, 70124 Bari, Italy; f. Pharmaceutical Department, Local Sanitary Unit Umbria 1, 06132 Perugia, Italy; g. Microbiology and Virology Unit, Department of Interdisciplinary Medicine, School of Medicine, University of Bari ALDO MORO, 70124 Bari, Italy; h. Department of Biology, College of Science and Technology, Temple University, Philadelphia, PA 19122-6078, USA.

Abstract: Oxidative stress plays an important role in brain aging and in neurodegenerative diseases. New therapeutic agents are necessary to cross the blood–brain barrier and target disease pathogenesis without causing disagreeable side effects. Resveratrol (RSV) may act as a neuroprotective compound, but little is known about its potential in improving the cognitive and metabolic aspects that are associated with neurodegenerative diseases. The objective of this study was to investigate the protective effects and the underlying mechanisms of RSV against hypoxia-induced oxidative stress in neuronal PC12 cells. For the induction of the hypoxia model, the cells were exposed to oxygen deprived gas in a hypoxic chamber. Cell cycle and apoptosis were analyzed by a fluorescence activated cell sorting (FACS) analysis. The intracellular reactive oxygen species (ROS) level was analyzed by using dichlorodihydrofluorescein diacetate (DCFDA) and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA) tests. The expressions of activated caspase-3, -9, Bcl-2, Bax, p53, and SOD were investigated by a Western blot analysis. We found that hypoxia reduced PC12 viability by inducing apoptosis, while RSV treatment attenuated the ROS-induced damage by reducing caspase-3, -9, and the Bax/Bcl-2 ratio. The RSV treated groups were found to improve cellular health, with a 7.41% increase in the S phase population in the 10 µM group, compared to the control. Hence, RSV has a protective effect in neuronal cells and may halt the cell cycle in the G1/S phase to repair the intracellular damage. Therefore, RSV could be a good candidate to act as an antioxidant and promising preventive therapeutic agent in neurodegenerative diseases for personalized medicine.

P04. Molecular Characterization Of Stem Cells Isolated From Human Gastric Organoids To Study Stomach Pathophysiology

Author(s): Chiara Balzamo[1], Elena Amendola[2], Valeria Lucci[1], Francesco Albano[3], Giovanni Calice[3], Pellegrino Mazzone[4], Giuseppina di Paola[4], Simona Laurino[3], Luigi Pasquale[5], Desiree Picascia[5], Andrea Fiorentino[5], Alessandro Di Sarno[5], Simona Ricciolino[5], Geppino Falco[1]

Institution(s): 1 Department of Biology, University of Naples Federico II, 80126 Naples, Italy. 2 Institute of Experimental Endocrinology and Oncology "G. Salvatore", National Research Council (CNR), via Pansini, 5, 80131 Naples, Italy. 3 IRCS CROB-Referral Cancer Center of Basilicata, 85028 Rionero in Vulture, Italy. 4 Biogem Scarl, Istituto di Ricerche Genetiche "Gaetano Salvatore", Ariano Irpino, Italy. 5 Gastroenterology and Digestive Endoscopy Department, O. Frangipane Hospital, Ariano Irpino (AV), ASL Avellino, Italy.

Abstract: Human adult gastric stem cells (hAGSCs) are essential for regulating the physiological turnover of the gastric epithelium and damage repair, due to their self-renewal capability, high proliferating rate and multipotency. Moreover, the study of hAGSCs allows the understanding of stomach pathological events, such as precancerous lesions and gastric cancer [1]. In the last decades several studies in mice have identified different populations of AGSCs, however the knowledge about the molecular identity and plasticity of hAGSCs is still limited, because of the lack of markers that facilitate their isolation [2-3-4-5-6-7]. Progastricsin (PGC) and aquaporin 5 (AQP5) mark two hAGSC populations in different anatomical regions of the stomach: *corpus* and *antrum*, respectively. In addition, mucin 6 (MUC6) and the alpha-1,4-N-acetylglucosaminyltransferase(A4GNT) present enriched expression in human pyloric AQP5-expressing population in the *antrum* [2-8]. Our goal is to isolate and characterize human PGC^+ *corpus* and $AQP5^+$ *antrum* stem cells in patient-derived organoids (PDOs). For this purpose, weevaluated the expression and cellular localization of PGC andAQP5, MUC6 and A4GNT in PDOs and biopsies by RT-PCR and immunostaining, confirming that they display an expression consistent with their expected role as markers of gastric stem cells of the *corpus* and *antrum* respectively. Cellular isolation by endogenous protein is often difficult, due to the poor accessibility of certain antigens and/or the reduced number of marker-expressing cells. For this reason we will engineer the PDOs with the green fluorescent protein under the control of the minimal promoter of PGC, AQP5, MUC6 and A4GNT that will allow us to isolate the corresponding cytotypes by cytofluorometry.
Finally, our study will prompt new insights into gastric stem cell biology and open up a new window into gastric cancer biology, also providing developmental opportunities in personalized and regenerative medicine.

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P05. MiR423-5p/Malat-1 loop as a new tool for therapeutic intervention in Hepatocarcinoma

Author(s): Marco Bocchetti[1,3,5],Carmela Ferri[2], Alessia Maria Cossu[1,3], Federica Melisi[1,3], Piera Grisolia[1,3], Marianna Scrima[3], Mosab Jedea[1], Amalia Luce[1], Nicola Amodio[4], Tarik Regad[5], Silvia Zappavigna[1], Michele Caraglia[1]

Institution(s): 1 Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. De Crecchio, 7, 80138 Naples, Italy. – Italy.
2 Medicina Futura Group, Coleman S.P.A, Via Alcide De Gasperi 107/109/111, 80011 Acerra (NA), Italy. – Italy. 3 Laboratory of Precision and Molecular Oncology, Biogem Scarl, Institute of Genetic Research, Contrada Camporeale, 83031 Ariano Irpino, Italy. – Italy. 4 Department of Experimental Medicine, University of Catanzaro "Magna Graecia", 88100, Viale Europa, Catanzaro, Italy – Italy. 5 The John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Clifton Lane, NG11 8NS, Nottingham, United Kingdom. - United Kingdom

Abstract: Non-coding RNAs, such as micro-RNAs and long non-coding RNAs, are not translated into proteins but still heavily modulate cellular behavior. We recently found that micro-RNA 423-5p is overexpressed in serum of hepatocellular cancer (HCC) patients responding to Sorafenib, inducing autophagy in HCC models. This could be due to the interaction with oncogenic lncRNA Malat-1, in silico miR423-5p predicted target. For a deeper insight into that, we generated stable overexpressing HCC clones using Lentiviral Transduction system. At first, miR423-5p and Malat-1 interaction was confirmed by RIP assay then we studied proliferation, migration, invasion and clonogenicity potential with different techniques. MiR423-5p+ cell lines had reduced levels of Malat-1 and were struggling to proliferate, migrate and invade compared to controls, while Malat-1+ clones showed the opposite phenotype. Mass Spectrometry and other observations suggested a reduced level of mitochondrial proteins in miR-423-5p transduced cells, pushing us to investigate the mitochondrial activity. Confocal microscopy evidenced miR423-5p+ cell lines to have a reduced number and smaller mitochondria compared to parental cells, while opposite effects were recorded in Malat-1+ cells. Interestingly, we found most of the mitochondrial-related genes, directly correlated to cell energetic and metabolic activity, to be downregulated in miR423-5p+ clones and upregulated or unmodified in Malat-1+ clones. Furthermore, we performed NGS Analysis on Long Non-Coding RNAs and whole transcriptome, with interesting and fitting results. Experiments on Liver Cancer Organoids are in progress in order to confirm our previous findings on a more appropriate and closer to clinic model. Finally, we tested a novel miRNA nanoparticle-delivery based method exploiting autofluorescent Nanodiamonds, which appeared to be a promising strategy both in vitro and in vivo. Our results offer an exciting starting point to be further investigated for innovative precision medicine approaches, possibly exploiting miR423-5p as oncosuppressor.

P06. A pilot study of miRNA expression profile in surgicallyresected pancreatic ductal adenocarcinoma: initial report from a bi-institutional cohort

Author(s): Carlo Caputo[a], Michela Falco[a], Giuseppe Tirino[b], Marco Montella[c], Anna Grimaldi[a], Gabriella Misso[a], Angela Lombardi[a], Madhura Tathode[a], Ferdinando De Vita[b], Michele Caraglia[a].

Institution(s): [a]University of Campania Luigi Vanvitelli, Department of Precision Medicine, Division of Molecular Pathology, Naples, Italy. [b]University of Campania Luigi Vanvitelli, Department of Precision Medicine, Division of Medical Oncology, Naples, Italy. [c]University of Campania Luigi Vanvitelli, Department of Mental and Physical Health and Preventive Medicine, UOC Pathological Anatomy, Naples, Italy.

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic malignancy characterized by poor prognosis, even for resectable patients (pts). Numerous studies have demonstrated the role of miRNAs in the regulation of gene expression by targeting the mRNAs involved in cancer processes, suggesting their use as clinical biomarkers. We performed a microarray analysis of miRNA expression profile from surgical tissues collected from 20 resected PDAC pts pooled into 4 groups according to different clinicopathological features: nodal metastases (N+/N-) and tumor grading (G3/G2). So, 11 miRNAs were significantly modulated in G3 vs G2 analysis and 7 in N+ vs N- comparison, suggesting a possible specific signature reflecting histological grade and nodal metastasis occurrence, respectively. The expression of 3 up-regulated miRNAs (miR-1-3p, miR-31-5p and miR-205-5p), that emerged from the G3 vs G2 comparison was validated on the tissues of 11 pts PDAC grade G3 and 5 pts PDAC G2. The results showed a significant up-regulation of miR-1-3p and miR-31-5p in G3 pts compared to G2 pts, but not for the miR-205-5p. Focusing on the validated miRNAs (miR-1-3p and miR-31-5p) in PDAC grade G3 pts, we carried out the prediction assays to identify putative miRNA target genes by three computational strategies. So, overlapping miRNA target genes analysis (predicted and experimentally validated) we identified 9 genes as common targets regulated by both miRNAs expressed in tumor grade conditions. Among these identified targets, we would like to focus on PAX5 gene, (common targets of both validated miRNAs), not yet validated to study its involvement in multiple signaling pathways commonly dysregulated in cancer. A further validation will be performed in tissue samples and in *in vitro* 3D organoids models of pancreatic adenocarcinoma. The patient-derived organoids (PDOs) will be useful to test different treatments as well as to study the underlying molecular causes of cancer and treatment resistance.

P07. GSTEP Organoids Research Core Facility

Author(s): Eleonora Cesari[1], Alessandra Ciucci[2], Giovanni Scambia[2,3] and Claudio Sette[1,4]

Institution(s): [1]GSTEP Organoids Research Core Facility, IRCCS Fondazione Policlinico A. Gemelli, 00168 Rome, Italy; [2]Department of Woman and Child Health and Public Health, Catholic University of the Sacred Heart, 00168 Rome, Italy;
[3]Gynecologic Oncology Unit, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Largo Francesco Vito 1, 00168 Rome, Italy; [4]Department of Neuroscience, Section of Human Anatomy, Catholic University of the Sacred Heart, 00168 Rome, Italy;

Abstract: The “Gemelli Science and Technology Park” (G-STeP) project is part of a broad research program set up by “IRCCS Fondazione Policlinico Universitario A. Gemelli”. This program is aimed at enhancing, connecting and structuring all the research activities that take place at the Foundation and in collaboration with other Hospital or Research Institute. It is for this reason that G-SteP was born as a network of services to support all stages of development of a scientific research project. The G-STeP is made up of over 20 Research Core Facilities that offer research services divided by technological topic and capable of providing specific services with certified quality. Access to services is possible through the G-step app [1], a specially created application through which researchers will have direct and rapid access to the services.
One of these Facilities is the Organoids Facility. Patient derived organoids can predict the response of patients to treatments and may therefore guide therapeutic decisions. Although preliminary results appear encouraging, organoids still need to be generated and expanded efficiently to enable drug screening in a clinically meaningful time window. A new generation of clinical trials based on the organoid technology should guarantee tailored approaches to cancer management, as it is now clear that the one-size-fits-all approach cannot lead to efficient and meaningful therapeutic advancements. This is precisely the purpose of our facility that is actually involve in several project in different fields of cancer research: High Grade Serous Ovarian Cancer (HGSOC), Breast Cancer and Pancreatic Ductal Adenocarcinoma.

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P08. P2X4 receptor antagonist induces cell death throughalteration of mitochondria membrane potential and Ca^{2+} entry in 3D model of renal carcinoma.

Author(s): Filomena de Nigris[1], Giulia Ciampi[1] , Angela Giliberti[1].

Institution(s): 1 Department of Precision Medicine University of Campania L. Vanvitelli, Naples, Italy.

Abstract: Clear cell renal cell carcinoma (ccRCC) is the most common (75%) lethal subtype of kidney cancer. Large-scale metabolomics data have already connected metabolism to the pathogenesis and/or progression of kidney cancer and correlated mitochondria activity with patients' poor survival. Here, we show that oxophosphorylation is the main source of tumor-derived ATP, which exerts a critical impact on tumor energy metabolism. We show that the oxygen consumption rate from the mitochondrial activity was greater in renal carcinoma cell lines and patients than in glycolysis activity. Mitochondrial activity depends on intracellular calcium provided in large part by the lysosomal P2X4R receptor. Seahorse experiments showed that the P2X4Rantagonist caused a rapid dose-dependent reduction of both intracellular calcium and mitochondrial activity. The prolonged mitochondrial failure increased radical oxygen species changes in mitochondrial permeability opening the transition pore complex (PTPC), dissipating the mitochondrial membrane potential (Δψm) and calcium overload, and finally, cell death via both necrosis and apoptosis. These results in some renal carcinoma cell lines in 4 renal carcinoma organoids, in xenograph and renca mice model, indicate that the balance between calcium and mitochondrial activity presents an opportunity to target renal carcinoma.

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P09. Targeting cancer cell motility to prevent metastasis in colorectal cancer

Author(s): Donatella Delle Cave[a], Annalisa Di Domenico[a],Marianna Ciotola[a], Ilaria Di Biase[a], Enza Lonardo[a]

Institution(s): [a]Institute of Genetics and Biophysics Adriano Buzzati-Traverso (IGB), National research Council (CNR), via pietro castellino 111, Naples (Italy)

Abstract: Mortality and morbidity in patients with colorectal cancer (CRC) consistently results from the disruption of physiological function triggered by disseminating cancer cells (DCCs). Cancer cellmotility is accounted as the primary reason of metastasis. The prerequisite for cancer cell motility has been provenexperimentally by pre-clinical studies involving a large set of migration-associated factors. Yet, there are scarce clinical approaches planned to precisely target the cancer cells motility and to exactly counteract cancer cell dissemination. Transforming growth factor-β (TGF-β) signaling is involved in CRC metastasis, thus leading to a dismal prognosis of patients. Interestingly, TGF-β pathway may promote tumor progression by regulating DCCs behavior, but the mechanism underlying is still debated. By using patients-derived-organoids (PDOs) we found that DCCs are characterized by increased expression of L1CAMand CXCR4, both induced by the presence of Nodal and TGF-β1 in the microenvironment. L1CAM^{high}/CXCR4^{high} PDOs showed migrating properties, resistance to conventional therapy, and *in vivo* forming liver metastasis, compared to the negative counterpart. To eradicate the L1CAM^{high}/CXCR4^{high} population we developed two strategies: 1) Inhibiting of TGF-β1 and Nodal receptors with SB431542 molecule alone or in combination with the standard chemotherapeutic agent 5-fluorouracile (5-FU) [1]; 2) Delivering the Galunisertib (Gal), an inhibitor of TGF-βreceptor I, by using biosilica nanoparticles (NDP-Gal) [2]. We found that TGF-β1/Nodal inhibition sensitized L1CAM^{high}/CXCR4^{high} cells to 5-FU reducing their potential to give rise liver metastases. Moreover, we observed that the NDP-Gal were efficiently internalized in the CRC-PDOs decreasingtheir migratory potential and stem properties. In conclusion, we demonstrated that a subpopulation of DCCs is characterized by elevated expression of L1CAM and CXCR4, two factors involved in cancer cell motility and metastasis formation. Strategies aimed at the eradication of this population, by targeting the TGF-β pathway, will drastically reduce the tumorigenic and metastatic potential of CRC cells.

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P10. Expansion of lung adenocarcinoma stem cells rely on ALDOC- and ENO2-driven glucose metabolism regardless of nutrient environmental conditions.

Author(s): Claudia De Vitis[a*], Flavia Biamonte[b],Matteo Pallocca[c], Valentina Salvati[d], Gianluca Santamaria[b], Anna Martina Battaglia[b], Alessandro Sacco[b], Giulia Ricci[e], Maurizio Fanciulli[f], Ferdinando Chiaradonna[g], Gennaro Ciliberto[d], Rita Mancini[a].

Institution(s): [a] Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, "Sapienza" University of Rome, Italy; [b] Department of Experimental and Clinical Medicine, "Magna Graecia" University of Catanzaro, Italy [c]Biostatistics, Bioinformatics and Clinical Trial Center, IRCCS Regina Elena National Cancer Institute, Rome, Italy; [d] Scientific Director, IRCCS "Regina Elena" National Cancer Institute, Rome, Italy; [e] Department of Experimental Medicine, Università degli studi dellaCampania “Luigi Vanvitelli”, Naples, Italy; [f] SAFU Laboratory, IRCCS Regina Elena National Cancer Institute, Rome, Italy; [g] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy;

Abstract: Cancer metabolism has traditionally been equated with the Warburg effect {Citation} [1]. the mutations in oncogenes and loss-of-function alterations of oncosuppressors increase the metabolic demands of cancer cells and require an adaptive response to ensure cell fitness, interfering with intracellular signalling and gene expression [2]. 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. 3D spheroids derived from primary cell cultures derived from malignant pleural effusion (MPE) and stable cell lines lung adenocarcinoma (LUAD) were cultured in either nutrient-rich or -restricted culture conditions. A multi-omics approach 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 lung cell lines. We found that the transition from 2D to 3D cultures 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, cell viability, but also the number and size of spheroids produced by cell lines. 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. The pan-cancer validation in primary cell culture from MPE of this vulnerability could potentially help to slow or prevent cancer progression.

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P11. Flavonolignans as modulators of P-gp mediated multidrug resistance

Author(s): Simona Dobiasová[a], Tomáš Nejedlý[a], Martina Hurtová[a,b], David Biedermann[b], Kateřina Valentová[b], Vladimír Křen[b], Jitka Viktorová[a]

Institution(s): [a] Department of Biochemistry and Microbiology, University of chemistry and technology in Prague, Czech Republic [b] Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Abstract: The long-term action of the chemotherapeutics on a malignant tissue supports the emergence of mechanisms that reduce the sensitivity of cells to the administered treatment. The mechanism of increased activity of ABC transporters (e.g. P-glycoprotein (P-gp)) is among the most frequently correlating factors in MDR development. One of the possibilities for an effective solution is the enhancement of cytostatic effectiveness, thanks to their combination with transporter inhibitors. New generation of P-gp modulators is complemented by natural compounds as flavonolignans [1]. Hydnocarpin flavonolignans have shown remarkable biological effects in tests on 2D models, including resistance modulation in ovarian cancer. The testing of flavonolignan potential (hydnocarpin, hydnocarpin D, hydnocarpin D benzoate, izohydnocarpin) was associated with a cytoxicity monitoring on ovarian cancer cell line (A2780 and its doxorubicin-resistant subline) and control non-tumor cell lines(human skin fibroblasts (HDF), primary renal tubular epithelial cells (HRTEC)). Hydnocarpin and hydnocarpin D showed a higher degree of cytotoxicity, which was reflected in a lower inhibitory concentrations (IC_{50}) in all lines. The direct impact on P-gp modulation was observed by all mentioned compounds. Each of the samples was able to inhibit P-gp in dose-dependent manner, resulting in a decrease of ATP consumption in luciferase reaction. Hydnocarpin flavonolignans confirmed the ability to sensitize a doxorubicin-resistant ovarian cells(A2780/ADR), resulting in an enhanced cytostatic effect. The expansion of knowledge about the effect of substances will be testing their potential on 3D cell models (spheroids), which represent a significant step forward to *in vivo* conditions.

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P12. Anti miR-223 as novel therapeutic strategy: opportunities and challenges in Laryngeal Squamous Cell Cancer

Author(s): Michela Falco*[a,c], Chiara Tammaro*[a], Alessia Maria Cossu[a,c] , Amalia Luce[a], Elisabetta Verderio Edwards[d], Giuseppe Scafuro[a], Sara Martino[a], Amough Auti[a], Filippo Ricciardiello[b], Teresa Abate[b], Giuseppe Longo[b], Giovanni Motta[d], Gaetano Motta[d], Michele Caraglia[a,c], Gabriella Misso[a]

Institution(s): a. University of Campania "Luigi Vanvitelli", Department of Precision Medicine, Naples, Italy. b.Cardarelli Hospital, Department of Ear Nose and Throat Unit, Naples, Italy. c. BIOGEM scarl Institute of Genetic Research, Ariano Irpino, Italy. dUniversity of Campania "Luigi Vanvitelli", Department of Ear Nose and Throat Unit, Naples, Italy. e School of Science and Technology Nottingham Trent University
*These authors contributed equally to this work

Abstract: Conventional methods such as surgery, radiotherapy and chemotherapy are the gold standards for LSCC treatment, but due to the negative impact on patient's quality of life, novel targeted therapy approaches with lower side effects are coveted. Moreover, because of tumor stage remains a prominent factor for treatment planning. useful prognostic factors. Recently, circulating microRNAs have been evaluated for their potential role as biomarkers or therapeutic targets. the absence of Our study displayed a significant upregulation of miR-223 in both sera and tissues of LCa patients compared to healthy samples. In addition, we highlighted a positive correlation of miR-223 with tumor stage. This evidence encouraged our investigation of the biological role of miR-223 and its antagomir on in vitro LSCC model generated by a lentiviral transduction of Hep-2 cell line. As a result, miR-223 stable expression seemed to increase viability, clonogenicity and migratory capacity, while an opposite effect on the same processes was underlined by the constitutive expression of miR-223 antagomiR. In addition, we broadened our research on this promising miRNA, deeply investigating, by quantitative proteomic analysis, the molecular pathways affected by miR-223. In detail, we performed an expression analysis focusing on the proteins involved in cancer progression processes, metastasis, and epithelial-to- mesenchymal transition (EMT). Moreover, we also simultaneously profiled 48 specific EMT genes by using a TaqMan Array plate platform. These assays confirmed our hypothesis of a higher aggressive profile conferred to laryngeal cancer cells by miR-223 overexpression. In this scenario miR-223 could represent a promising prognostic biomarker and its inhibitor an interesting therapeutic agent. To this end, the establishment of self-organized three-dimensional tissue cancer organoids from patients' tumors is ongoing. Their growth and response to conventional drugs and/or miR-223 inhibitor will be characterized with the aim of predicting in vivo drug sensitivity to develop a precision treatment for LSCC.

P13. Generation of non-small cell lung cancer organoids from hPSCs derived from human cell lines

Author(s): Martina Godel 1, Joanna Kopecka [1], Chiara Riganti[1]

Institution(s): [1] Department of Oncology, University of Torino, via Santena 5/bis, 10126 Torino, Italy

Abstract: Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancer cases. Surgical removal remains the treatment of choice for early stages, but with a late diagnosis the main therapeutic options reside in chemotherapy and targeted therapies. Unfortunately, up to 60% patients develop drug resistance. Many reasons that underlie resistance to therapy have their roots in the heterogeneity of the tumor itself [1]. As 2D cell cultures do not quite recapitulate the tumor complexity, the aim of our work was to generate a suitable 3D experimental *in vitro* model to study drug resistance in NSCLC. Patient-derived organoid cultures of NSCLC, which would be the ultimate representative *in vitro* model, are currently difficult to standardize. In keeping with this, we generated organoids from human pluripotent stem cells (hPSCs) of two commercially available NSCLC cell lines (A549, Calu-3) and of one non-transformed human bronchial epithelial cell line (Beas-2B), as control. Firstly, we induced the de-differentiation of commercial cell lines by culturing them in suspension and in media supplemented with growth factors inducing stemness-stimulating pathways. After two weeks, we checked the presence of hPSCs by assessing lung stem cells markers (SOX2, CD44, CD133). Next, we differentiated hPSCs into NSCLC organoids, using FGF10 [2], and checked differentiation markers. As read out assay, we measured the response to chemotherapeutic drugs (cisplatin and pemetrexed), compared to parental 2D cell lines.
This work successfully generated organoids from commercially available NSCLC cells lines, exploiting de-differentiation to hPSCs and re-differentiation into 3D tumoral structures, which can be used to assess drug resistance and perform drug screening.

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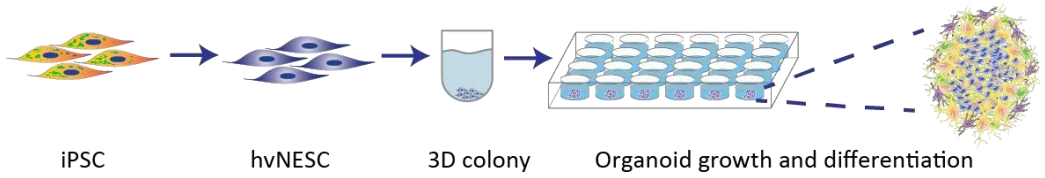
P14. 3D brain organoid models as a tool to screen for nutraceuticals

Author(s): Arianna Minoia[1], Luca Dalle Carbonare[1*], Jens C Schwamborn[2], Silvia Bolognin[2] and Maria Teresa Valenti[3*]

Institution(s): [1]Department of Medicine, University of Verona, University of Verona, 37100 Verona, Italy [2]Luxembourg Centre for Systems Biomedicine (LCSB), Developmental and Cellular Biology, University of Luxembourg, 4365 Belvaux, Luxembourg [3] Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, 37100 Verona, Italy

Abstract: Degenerative conditions of the skeleton and the brain are significant issues with significant socioeconomic effects. This study would be underlines the significance of the interaction between nerve and bone cells 3D brain organoid models as a tool to screen for nutraceuticals which have an impact on skeletal metabolism. Knowing that one of the main and important pathways that links bone metabolism and the brain is the Wnt / β-catenin pathway and plays a crucial role in the development of many aspects of midbrain DA development, we would try to understand the effect of some molecules chronically treating 3D organoids derived from two different cell line, for up to 40 days. Focusing on the study of genes involved in the Wnt / b-catenin pathway and in neuronal degeneration.

Material and methods



iPSC	hNESCs WT/GC hNESCs MUT	Organoids treat with different compounds for up 40 days

Results

We investigated the gene expression of PARK2, NR2F1, CTNNB1, and LRP5 for 3D organoids treated with DMSO, lipoic acid, and JH-II in two different cell lines characterized byLRRK2-G2019S mutation and without the mutation (wt/gc). The molecules impact on the expression of the genes associated with the Wnt /β-catenin pathway upstream and downstream appear to have a beneficial impact on the rise in the expression of the target genes. Considering an improvement in the conditions of PD patients, the treatment with the molecules we examined would result in increase the expression of the genes of our interest.

P15. The preparation of 3D cell models for testing silybin flavonolignans as multidrug resistance modulators

Author(s): Tomáš Nejedlý[a], Simona Dobiasová[a], Martina Hurtová[a,b], David Biedermann[b], Kateřina Valentová[b], Vladimír Křen[b], Jitka Viktorová[a]

Institution(s): a Department of Biochemistry and Microbiology, University of chemistry and technology in Prague, Czech Republic b Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Abstract: One of the major problems in cancer therapy is the development of multidrug resistance (MDR). MDR can be caused by the overexpression of ABC family efflux pumps, especially P-glycoprotein (P-gp). Due to higher P-gp activity, cytostatics are pumped out of the cells, which cause a low chemotherapy efficiency. To overcome MDR by blocking P-gp mediated efflux, bioactive compounds from natural sources are used [1]. For instance, flavonolignans from Silybum marianum seem to be the part of next generation of natural P-gp modulators. The aim of this study is to optimize the preparation of 3D cell models (spheroids) and compare MDR modulation effect of silybin flavonolignans in 2D and 3D models. To compare the activity, the different cancer cell lines were selected – human ovarian (HOC) and breast (MCF-7) cells with the corresponding drug- resistant sublines. Firstly, different methods of spheroid preparation were used – hanging drop and liquid overlay technique. The compact ovarian spheroids (HOC; sensitive line and its doxorubicin-resistant subline) were prepared only by the hanging drop technique, due to the reduced aggregation rate of cells. In case of breast cancer (MCF-7; sensitive line and its paclitaxel resistant subline), compact microtumors were prepared by both techniques. Subsequently, the cytotoxic potential of flavonolignans was evaluated. 3D models showed higher resistance to the cytotoxic effect of silybin AB and dehydrosilybin AB compared to 2D models. Further studies will be focused on the verification of MDR modulation activity on 3D models.

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P16. Role of ADP-ribosylation in breast cancer sensitization to apoptosis: PARP12 as a novel therapeutic target

Author(s): Anupama Pavithran[a*], Angela Filograna[a], Nina Dathan[a], Matteo Lo Monte[a], Daniela Corda[a] and Giovanna Grimaldi[a]

Institution(s): [a]Institute of Endocrinology and Experimental Oncology “G. Salvatore”, 2nd unit, National Research Council, Naples, Italy

Abstract: Breast cancer is one of the most prevalent cancers worldwide where chemotherapy can be used in an integrative approach. However the chemotherapeutic benefits are transient, identified by the lack of tumor response due to chemotherapeutic resistance shown by the cancer cells. The culture of tumor organoids derived from breast cancer can aid us in understanding the underlying mechanisms that lead to chemotherapeutic resistance [1]. Our study focus to investigatethe mechanisms that lead to chemotherapeutic resistance, with a vision to provide survival benefits to affected patients through targeted combination therapies. On this note, PARP12, a mono-ADP-ribosyltransferase of the PARP family has been identified as a key factor in breast cancer resistance to chemotherapy by contributing to tumour survival and re-growth [2]. To evaluate this further, we studied the PARP12 depletion effects in several cancer cell lines of different origin. Significant results demonstrated that transient depletion of PARP12 promotes apoptosis selectively in breast tumoral cells, as detected by FACS analysis and PARP1 cleavage. Interestingly, at molecular level we found that Akt, a major regulator of cell survival [3], is a PARP12 substrate. Further, by exploiting bioinformatic approaches, putative Akt ADP-ribosylation defective mutants have been identified . We have validated a specific set of residues located in the kinase domain to be affecting the catalytic activity of Akt when mutated thus demonstrating that MARylation of Akt is functional to sustain its kinase activity. A functional analysis of how this mutant responds to apoptosis specifically focusing on PI3K/AKT/mTOR pathway is currently our interest. Elucidating how MARylation of Akt by PARP12 sensitise the cancer cells to apoptosis will be instrumental in defining the role of PARP12 in breast cancer resistance to chemotherapy. Subsequently; evaluating PARP12 deficiency in organoids will serve as an integral component of the pipeline for the discovery of the role of PARP12 in breast cancer resistance to chemotherapy.

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P17. Head and neck cancer organoids as a tool to predict therapeutic efficacy of a novel anti- PDL-1 Fab

Author(s): Ylenia Ferrara[1], Annachiara Sarnella[1], Debora Latino[1], Menotti Ruvo[1], Annamaria Sandomenico[1], Antonella Zannetti[1]

Institution(s): 1. Instituto di Biostrutture e Bioimmagini-CNR, Naples, Italy

Abstract: The head and neck squamous carcinomas (HNSSC) are placed at the sixth place in the world for incidence among all solid human malignancies. Despite the availability of more advanced chemo- radiation and surgical treatments, mortality for HNSSC remained virtually unchanged due to their resistance to therapies [1]. Recently, our studies reported the importance of hypoxic tumor microenvironment in promoting resistance to cisplatin in HNSCC cells and how the inhibition of carbonic anhydrase IX could sensitize them to the chemotherapeutic treatment [2]. Checkpoint inhibitors, such as PD-1/PDL-1 inhibitors, have demonstrated clinical efficacy in many cancers including HNSCC. Anti-PDL-1, Atezolizumab demonstrated tolerability and encouraging clinical activity in a heavily pretreated head and neck cancer cohort [3]. The purpose of this study was to characterize and test a novel antibody-drug conjugate (ADC), anti-PDL-1 Fab, which will be linked to a chemotherapeutic agent, in HNSCC cell lines and organoids. First, PDL-1 expression levels were analyzed in FaDu and SCC-011 cells by western blotting, and very high levels were observed in both HNSCC cell lines. Binding of anti-PDL-1 Fab to FaDu and SCC-011 cells was evaluated using FITC secondary antibody and fluorescence-activated cell sorting (FACS). Parallel experiments were performed using FITC-Atezolizumab as a control. Both anti-PDL-1 antibodies specifically bound to PDL-1 expressed on FaDu and SCC-011 cells. To better reproduce the tumor response to drugs, it is necessary to consider the support of the tumor microenvironment. Therefore, we developed and established 3D organoids from tissues obtained from HNSCC patients. The binding of anti-PDL-1 Fab to HNSCC organoids was evaluated as described above, and specific binding of about 60% was found. In conclusion, our results demonstrated the ability of this novel Fab to bind specifically to PDL-1 in 2D cells and organoids of HNSCC, making it a promising ADC for the treatment of HNSCC.

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