PhD Days 2018
PhD Programme in Molecular Life Science
Aula Magna, Università degli studi della Campania "Luigi Vanvitelli"

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Chimeric Recombinant Antibody Fragment of anti-Nodal 3D1 and anti-Cripto 1B4 for theranostic applications
Tutor: Menotti Ruvo and Luigi Vitagliano

Nicola Landi
Ribotoxin-like proteins in edible mushrooms: purification, characterization and their possible cytotoxic activities
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Giovanni Mastroianni
Untargeted Metabolomics evaluation of nutraceuticals using NMR as main analytical platform
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Miguel Moreira
Structural and functional insights into complement modulation by the complement regulatory protein CD55
Tutor: Rita Berisio and Ruvo Menotti

Alessandra Monti
Development of inhibitors targeting AIF/CypA lethal complex
Tutor: Nunzianna Doti

Michela Napolitano
Mechanism of BARS-mediated mitotic Golgi fragmentation
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Odeta Celaj
Secondary metabolites from Mediterranean plants for nutraceutical and pharmaceutical applications
Tutor: Antonio Fiorentino

Sara Ragucci
Biological activities of the ribotoxin Ageritin from *Agrocybe aegerita*: a possible novel neurotoxin as tool to study nervous system model cells
Tutor: Antimo Di Maro

Valeria Sivo
Effects of Zn(II) replacement with Pb(II), Hg(II) or Ni(II) on the structure and function of prokaryotic zinc-finger domain
Tutor: Carla Isernia

Molecular Cell Biology

Alba Clara Fernández-Rilo
Obesity-Driven Neurodegenerative diseases: New insights for new molecular interplayers and therapeutic targets
Tutor: Luigia Cristiano

Simona Cataldi
Regulation of PPARγ signaling through alternative splicing and dominant negative isoforms
Tutor: Alfredo Ciccodicola

Maria Charalambous
Mitochondrial dynamics as a new therapeutic target for neurodegenerative diseases
Tutor: Prof..Dr. Lucio Nitsch

Chetan Dhakan
Molecular imaging of vulnerable atherosclerotic plaque in murine model using high frequency & contrast enhanced ultrasound
Tutor: Professor Silvio Aime

Giuseppe Delli Paoli
The saturation degree of fatty acids and their derived acylcarnitines determines the direct effect of metabolically active thyroid hormones on insulin sensitivity in skeletal muscle cells
Tutor: Pieter de Lange

Federica di Giacomo Russo
Molecular pathways activated by Excitatory Amino Acids in spermatogenesis
Tutor: Prof.ssa Chieffi

Maria Frola
Use of gene therapy for treatment of retina inherited dominant disorder
Tutor: Prof. Enrico Maria Surace

Francesco Paolo Iavarone
Cripto modulates angiogenesis and EndMT by controlling the shaping of pro-healing macrophages in skeletal muscle regeneration
Tutor: Gabriella Minchiotti

Concetta Iovine
In vivo evaluation of ellagic acid and curcumin effects in *Danio rerio* embryos
Tutor: Lucia Rocco

Jaipreet Singh Loomba
Role of Glycosphingolipid metabolic reprogramming in Neuronal Differentiation
Tutor: Giovanni D'Angelo

Federica Liccardo
Novel fluorescent probes for precision labeling in super-resolution microscopy
Tutor: Alberto Luini

Maria Mangini
sPLA2-IIA regulates osteoclast differentiation and function
Tutor: Stefania Mariggiò

Mehuli Chakraborty
Targeting the cancer (stem) cells – tumor microenvironment crosstalk to improve pancreatic cancer prognosis
Tutor: Enza Lonardo

Namrata Iyengar
Unravelling autoregulatory signalling circuits controlling export of different cargo classes from the ER
Tutor: DR. Alberto Luini

Pascale Emilia
A novel ultraconserved element containing long noncoding RNA is required to preserve transcriptional dynamics and maintain embryonic stem cell selfrenewal
Tutor: Annalisa Fico

Manpreet Pathej
Mechanism of Interaction of Glycerophosphoinositol and Shp-1
Tutor: Alessia Varone and Daniela Corda

Marta Panella
Mutual suppression of miR-125a and Lin28b in human hepatocellular carcinoma cells
Tutor: Prof. Aniello Russo

Rita Polito
Adiponectin and Immunity: This Adiponekine may be a specific biomarker for CVID disease?
Tutor: Prof.ssa AURORA DANIELE

Stefania Serpico
The Lysophosphatidic Acid Acyltransferase (LPAATs) Enzymes and their Role in Membrane Transport Alterations in Cancer
Tutor: Carmen Valente

Human Genetics

Ahmed El-Sharkawy
The interplay of NEMO, RIPK1 and RIPK3 signaling in the regulation of cell death
Tutor: Matilde Valeria Ursini

Rosita Del Prete
Characterization of murine models in imprinting disorders
Tutor: Alfonso Baldi

Jamal Naderi
Histone modification controls subcutaneous adipose tissue hypertrophy on the way towards type 2 diabetes
Tutor: Claudia Miele

Sabrina Napoletano
CRISPR-Cas9 neuronal cell model to investigate autophagy in neurodegeneration
Tutor: Emilia Vitale

Laura Pignata
Investigation of ICR2 epimutation in Beckwith-Wiedemann Syndrome
Tutor: Andrea Riccio

Antonella Rendina
Neuroimmune overlapping mutations leading to dementia: focusing on CD33 and TREM2 genes
Tutor: Emilia Vitale

Federica Scotto di Carlo
Dissecting the molecular mechanism underlying Paget's Disease of Bone complicated by Osteosarcoma
Tutor: Fernando Gianfrancesco

Lucia Verrillo
Towards the identification of new therapeutical compounds for a malignant epileptic encephalopathy caused by mutations in *Aristaless-related homeobox* gene
Tutor: Maria Giuseppina Miano

Yi-Shin Lee
Reactivation of the dormant wild-type allele of MECP2 as a therapy for Rett syndrome: screening of epigenetic compounds
Tutor: Marcella Vacca; Laura Casalino

Cancer biology, Immunology, Microbiology, Drug design

Alessia Ametrano
"Antarctized" antibody: an innovative engineered antibody by the CRISPR/Cas9 system
Tutor: Maria Rosaria Coscia

Ana Margarida Ferreira Campos
Modulating innate memory to treat inflammatory diseases
Tutor:

Deborah Cipria
Optimization of adoptive T cell therapy by promoting the correct pairing of T cell receptor chains
Tutor: Piergiuseppe De Berardinis

Barbara De Siena
Characterization of an efflux pump in *Mycobacterium smegmatis.*
Tutor: Lidia Muscariello

Giacomo Della Camera
New approaches to immunotherapy of inflammation through the use of engineered nanoparticles (ENP)
Tutor: Dr. Diana Boraschi

Daniela Esposito
COMET: a novel oncogenic long non-coding RNA that regulates MET in papillary thyroid carcinoma
Tutor: Valerio Costa

Federica Farina
Analysis of expression of HLA class II risk alleles in Celiac Disease
Tutor: Giovanna Del Pozzo

Valeria Gaudieri
Relationship between resistant hypertension and coronary vascular function with ^{82}Rb PET/CT in patients with suspected CAD.
Tutor: Wanda Acampa

Priyanka Gokulnath
Role of PAX8 in the Fallopian tube epithelium, the site of origin of HGSC
Tutor: Mariastella Zannini

Billy Samuel Hill
PDGFRβ as a new biomarker for metastatic triple-negative breast cancer: development of a theranostic anti-PDGFRβ aptamer for imaging and suppression of metastases
Tutor: Antonella Zannetti

Pietro Irrera
Development of MRI-based pH imaging as biomarker of treatment response.
Tutor: Dario Longo

Grant Garren January
"Exploitation of new strains for drug discovery from deep sea sediments"
Tutor: Donatella de Pascale

Magdalena Kostrzewa
Cannabinoids and bone: from metabolic to malignant diseases
Tutor: Dr. Fernando Gianfrancesco/ Dr. Alessia Ligresti

Narender Kumar
PD1 increases stemness and proliferation in Thyroid Cancer Stem Cell through Ras activation
Tutor: Prof. Rosa Marina Melillo

Ludovica Liguori
Pharmacological chaperones to cure genetic diseases
Tutor: Giuseppina Andreotti

Rita Lombardi
A proteomic approach identified HSP90 as a central hub in CDDP-resistant ovarian cancer cells.
Tutor: Dott. Alfredo Budillon

Ali Mokhtar Mahmoud
Cannabinoids as metabolic reprograming agents in prostate cancer cells
Tutor: Alessia Ligresti

Maria Rita Milone
Repurposing of valproic acid, simvastatin and aspirin combination as anticancer agents.
Tutor: Alfredo Budillon

Michele Minopoli
The Urokinase Receptor Antagonist RI-3 potently inhibits sarcoma cell invasion in a 3D organotypic co-culture model
Tutor: Maria Vincenza Carriero

Alejandro Moreiras Figueruelo
Drug-discovery from marine natural compounds
Tutor: Prof. Angelo Fontana

Olga pastorino
Glioblastoma: molecular compounds targeting tumor cells
Tutor: Prof. L. Colucci D'Amato

Pranoy Sahu
Identification and characterization of a Golgi glycosyltransferase as a new potential oncogene
Tutor: Prof. Alberto Luini; Dr. Riccardo Rizzo

Viera Laura Santana
Development of multifunctional RNA-based therapeutics to selectively target the stem-like glioblastoma cancer cells
Tutor: Vittorio de Franciscis

Marisa Saponaro
Identification of novel therapeutic strategies to treat neurodegenerative disorders
Tutor: Dott. Angelo Fontana; Dr. Genoveffa Nuzzo,; Dr. Carmela Gallo

Giovanni Andrea Vitale
Extreme environments as a source of new potential drugs
Tutor: Dr. Donatella de Pascale

Henu Kumar Verma
High-throughput Tissue based Molecular Classification of Human Gastric Cancer
Tutor: Geppino Falco

Session 1:

Gene Regulation and Computational Biology

Unraveling the molecular bases of genomic imprinting disorders: New KRAB-ZFP players in maintenance of DNA methylation

PhD student: Basilia Acurzio
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Imprinting Control Regions (ICRs) are genomic loci controlling the monoallelic and parent-of-origin expression of imprinted genes in placental mammals. Genomic imprinting is established at ICRs during gametogenesis by differential DNA methylation. Once acquired, the epigenetic marks are transmitted to the zygote and maintained in the embryo, despite the wave of genome-wide demethylation occurring in early development. The KRAB-Zinc Finger protein ZFP57 binds the methylated allele of the ICRs where it recruits the KAP1 complex, required for maintaining CpG methylation and H3K9me3 during preimplantation. Different studies have demonstrated conservation of the role of ZFP57 in maintaining DNA methylation between humans and mice.

Intriguingly, although ZFP57 null mouse ESCs show loss of methylation at all ICRs; ZFP57 mutant mice and human individual with homozygous ZFP57 mutation have only variable methylation defects of imprinted genes. These findings suggest that there may be functional redundancy in imprinting maintenance, perhaps because multiple KRAB-ZFP(s) bind the ICRs. To address this question, we are using different approaches that should allow to identify novel ZFPs involved in imprinting maintenance: one of these is the novel method Engineered DNA-Binding Molecule-Mediated Chromatin Immunoprecipitation (enChIP), combined with MS and NGS that will allow to identify proteins, RNAs and genomic regions interacting with the Kcnq1ot1:TSS ICR in mESCs.

Research and development of novel algorithms, methods and software tools for the integration and analysis of data produced by high throughput biological experiments

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In the last few decades, biological experiments produced a vast amount of data, especially using next-generation sequencing protocols. (NGS). NGS data are collected from different research experiments in context to the specific biological question in various format such as RNA-Seq, DNA-Seq, Chip-Seq, WGS, GWAS etc. So, many challenges exist to integrate and extract the useful information from these multi-omics data types. My PhD research aims to integrate useful biological information using in-house statistical approaches and computational methodologies. Specifically, we are interested in the development of an approach/pipeline for transcriptomics data integration produced by different high-throughput sequencing and array platforms. We are also a concern to study the evolutionary aspects of transcriptional rewiring due to differential splicing in

mammalian tissues as well as its role in the evolution of sexual maturation in ciliates.

1] The data produced by thousand of arrays and NGS experiments are perplexed with batch effects, i.e. systematic error introduced in the processing of samples in multiple batch or experiments. There is another complication appear, when different experimental setup adopts distinct protocols and technological platforms, to compare cross-platform gene expression data. In such scenarios, the regular differential expression analysis of genes is error-prone, and are not even feasible in those cases where data normalisation is not possible. To overcome this limitation, we are developing a computational pipeline with a graphical user interface Pipeline. It incorporates in-house build Python script for pre-processing, integration and external R packages for analysis of ranked gene expression data from multiple studies and for threshold independent investigation of strength, patterns and bound of correlation between ranked expression profiles from multiple cross-platform experiments.

2] In another collaborative work with Dr. Catania at the Institute for Evolution and Biodiversity at the University of Münster (Germany), We aim to investigate changes in gene expression levels and RNA processing profiles of ageing/sexually maturing Paramecia. These analyses will shed light on the molecular mechanisms that under lie sexual maturation in this single-celled eukaryote.

Effects of the 17-α-ethinylestradiol on Drosophila melanogaster

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17-a-ethinylestradiol (EE2) belongs to the increasing list of Endocrine Disruptors Chemicals (EDCs), used in the formulation of oral contraceptive pill and able to interfere with the endocrine system in both vertebrates and invertebrates. To date there is still little knowledge about its action mechanisms and harmful effects in invertebrates. To better evaluate its potential role in invertebrates, we used the model system Drosophila melanogaster, an insect in which the hormonal response has been widely described. The effects of EE2 in D.melanogaster adults have been evaluated by using life traits as well as molecular endpoints. It was found that EE2 significantly decreases survival and fertility in both sexes, with a higher effect in female flies, as well as affects the expression of the Ecdysone Receptor (EcR), Estrogen Related Receptor (ERR), Yolk protein2 (Yp2) and yolkless (yl) genes. In conclusion, our results suggest that EE2 treatment may have potential toxic and endocrine effects on Drosophila melanogaster adults of both sexes. In particular, our data provide an indication that, after EE2 treatment, two of the genes involved in the vitellogenesis process (yl and Yp2) are transcribed in adult males where are mostly silent, and suggest future studies forward their use as potential molecular markers to EDCs exposure in Drosophila male.

espressione estrogen receptor in males, what tissues show activation of Yolk proyein genes. why is survival decreased

Transcriptional and epigenetic deregulation of glycosphingolipid metabolism in Rett syndrome models

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Tutor: Floriana Della Ragione (floriana.dellaragione@igb.cnr.it)
PhD cycle: 32° cycle

Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked gene encoding the epigenetic factor Methyl-CpG-binding Protein 2 (MECP2). To date, the pathogenetic mechanism of RTT is still unclear. Correlations between RTT and glycosphingolipid (GSL) metabolism alteration were highlighted in brain of RTT patients. Interestingly, AUTS2, a transcription factor mutated in autism spectrum disorders, is coherently altered in brain of mice lacking or overexpressing MeCP2. AUTS2 promotes the expression of neuronal genes and, recently, we demonstrated that it regulates GSL metabolism driving neuronal differentiation.

Our data suggest that MeCP2 controls GSL metabolism through the binding of the promoter of GSL biosynthetic enzymes (GSEs) and modulates their expression in different brain regions. Moreover, MeCP2 and AUTS2 seem to regulate each other and both bind the promoter of ST3GAL5, encoding a key enzyme of ganglioside biosynthesis. Currently, MeCP2/AUTS2-mediated regulation of other GSE is under investigation by RNA-sequencing and qPCR. Furthermore, as we previously highlighted that globosides, GSLs poorly represented in neurons, negatively regulate MeCP2 and AUTS2 expression during neural differentiation, we generated mouse embryonic stem cells (mESCs) over-expressing the globoside Gb3. These cells will be used to decipher molecular mechanisms that link MeCP2 and AUTS2 to globosides in mESC-derived neurons.

Clustering for cell type identification in single cell RNA-seq data

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The classical approaches for determining cell types are mainly based on the morphological characteristics or expression of known markers. On the other hand, recent advances in single-cell RNA sequencing technology, allow for cell type identification based on the characterization of transcriptome - wide expression patterns. Through this approach, a large amount of sequencing data, reflecting transcriptome profiles of cells, are scanned to reveal biologically-relevant differences. However, due to the high level of noise present in “big data”, this task poses several challenges, that can only be handled with computationally efficient approach. Our proposed method for cell type identification from transcriptional data is mainly based on probabilistic mixture modeling. The model assumes the presence of latent cell populations in a mixture which are inferred with deterministic iterative algorithm. We demonstrate that the mixture based model in combination with nonlinear dimension reduction is superior to other widely used methods in terms of accuracy and computational time.

Integrated analysis of multi-omics single cell sequencing data

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Single cell sequencing is a powerful tool for exploring heterogeneity in cell populations. In recent years, there has been a shift in focus from bulk tissue analysis to the study of individual cells. Current sequencing technologies are capable of processing thousands of single cells at a time. Hence, there is a need for new and improved tools for processing and analyzing high throughput single cell data. During the course of my PhD project, I will develop algorithms, methodologies and software for the analysis of data obtained from various single cell multi-omic technologies. Towards this goal, I started with the analysis of publicly available single cell RNAseq datasets of different breast cancer subtypes. Cell lines and primary tumor cells are commonly used as models for understanding cancer mechanisms and tumor response to drugs. The primary aim of this study is to detect heterogeneity within single cell samples using isoform expression and alternative splicing events. This will allow us to study genes that are significantly spliced in different cancer models and the resulting pathways and biological functions that are affected. Future work will involve developing pipelines for an integrated approach to analyze multi-omics data.

Epigenetic landscape of D-amino acids system genes during development and in schizophrenia

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D-aspartate and D-serine modulate NMDA receptor-dependent transmission. Herein, we aimed to describe an epigenetic landscape of the genes modulating D-amino acids system evaluating the impact of expression of these genes on D-amino acids levels during development, and in neuropathological conditions like schizophrenia. D-aspartate content in the mouse brain decreases after birth, with concomitant increase of *Ddo* mRNA levels. Interestingly, postnatal *Ddo* gene expression is paralleled by progressive demethylation within its putative promoter. Embryonic cortical neurons treated with the DNA-demethylating agent, azacitidine, show a non-physiological increase of *Ddo* mRNA levels. Then we analyzed DNA methylation state and mRNA expression of the genes regulating D-aspartate and D-serine levels at different stages of mouse development, from birth to adulthood, in hippocampus, cortex and cerebellum. Our analysis revealed decreased *Ddo* gene methylation and specular increased mRNA levels in the hippocampus and cerebellum. Besides, *DAO* demethylation is associated with increased transcription in the cerebellum during development. Finally, we moved from mice to post-mortem brain of patients with schizophrenia and healthy controls. Epiallele classes and configuration analyses provided distinct area-specific patterns suggesting the occurrence of an orchestrated distribution of epialleles in diverse cell populations represented in each brain area, although no correlations were found with diagnosis.

in mouse, method for methylation analysis; how many CpGs, non-CpG methylation; methylation of adult tissues not expressing Ddo. in humans: methylation defines area, variability in humans

Development of novel computational tools, approaches and pipelines for the integration and analysis of high throughput transcriptomics data

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Tutor**:** Dott. Mario Rosario Guarracino (mario.guarracino@cnr.it)
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Affiliation: ICAR-CNR

I am working in a capacity of research fellow (Bioinformatician) at Institute for High- Performance Computing and Networking (ICAR), National Research Council, Naples, Italy, and also enrolled as a 3rd year PhD student in Molecular Biology, at Universita degli Studi della Campania Luigi Vanvitelli, Naples. During third year of my PhD research, I worked on several interesting projects related to the integration and analysis of high throughput transcriptomics data. In the first study, we device an integrated approach to understand these complex interactions. We analyze gene perturbation expression profiles, reconstruct a directed gene interaction network and decipher the regulatory interactions among genes involved in protein transport signaling. In particular, we focus on expression signatures of genes involved in the secretory pathway of MCF7 breast cancer cell line. These genes constitutes the endomembrane system, known as secretory pathway, is responsible for the synthesis and transport of protein molecules in cells. These genes are essential for the cellular development and function. Recent scientific investigations show that ER and Golgi apparatus may provide a convenient drug target for cancer therapy. On the other hand, it is known that abundantly expressed genes in different cellular organelles share interconnected pathways and co-regulate each other activities. The cross-talks among these genes play an important role in signaling pathways, associated to the regulation of intracellular protein transport. Furthermore, using network biology analysis, we delineate these gene-centric cross-talks at the level of specific modules/sub-networks, corresponding to different signaling pathways. We elucidate the regulatory connections between genes constituting signaling pathways such as PI3K-Akt, Ras, Rap1, calcium, JAK-STAT, EFGR and FGFR signaling. Interestingly, we determine some key regulatory cross talks between signaling pathways (PI3K-Akt signaling and Ras signaling pathway) and intracellular protein transport. In the second project, we focus on system toxicology approach to understand the mechanisms used by biological systems to respond to toxicants. Such understanding can be leveraged to assess the risk of chemicals, drugs, and consumer products in living organisms. In this work, we employ machine learning techniques and methodologies to develop prediction models for classification of toxicant exposure of biological systems. The outcome of this work is an experimental methodology to develop prediction models, based on robust gene signatures, for the classification of cigarette smoke exposure and cessation in humans. This work also provides gene signatures with top-ranked performances in the prediction of the investigated classification methods, as well as new discoveries in genetic signatures for biomarkers of the smoke exposure of humans. Apart from this, in 2018, I also work as a leading author on the development of novel computational tool to integrate and analyze functional genomics data such as Ranker: a graphical user interface for comparing expression profiles using rank based statistical approach. Results are going to be submitted in the journal paper. In this project, we carry out a comparative analysis of gene expression profiles, and develop a web-based interface "Ranker" employing Rank-Rank hyper-geometric overlaps (RRHO) and Prototype Rank List (PRL) data analysis methods. It includes four main features: (i) Conversion of expression data into rank matrix, (ii) prototype rank list generation, (iii) Distance calculation from PRL, (iv) RRHO analysis. Front-end interface of the Ranker is developed using in-house PHP, Java Scripts and HTML scripts. Core scripts for PRL based distance calculation and RRHO analysis is written in R and "Gene Expression Signature" and "RRHO" packages from Bioconductor are utilized. We presented this work in BBCC 2017 conference in Naples. In collaboration with R . Fazriyah, I am also working on the project to develop R package to test mean differences in gene expression data. The approach is based on alternative approach to test the significance of the difference between two means, when the sample size is small. This package does not require the hypothesis of homogeneity of variance between the two groups.

Altered DNMT3B functions in Immunodeficiency, Centromere instability and Facial anomalies syndrome cases (ICF1) syndrome

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Hypomorphic mutations in DNMT3B cause majority of ICF1 syndrome cases, resulting in immune response defects that cause premature death in childhood.
Through a transcriptomic and epigenomic study in patient-derived B-cell lines, our group recently contributed to clarify the altered mechanisms associated to the disease. DNMT3B dysfunction affects intragenic CpG methylation, thus impairing alternative TSS usage, antisense transcription and exon splicing. Interestingly, endogenous mutant-DNMT3B plays an active role in the perturbed exons splicing of the disease-relevant *CD45* gene, directly interacting with the pre-mRNA and heterogeneous ribonucleoproteins. Remarkably, a significant correlation between DNMT3B binding and exons inclusion/exclusion of mRNAs at genome-scale was found, implying a general mechanism to modulate mRNA alternative splicing.
In an attempt to identify potential factors involved in this regulatory process, we examined the 5mCprofile of the DNMT3B-interacting *CD45* transcript in patient and control B-cell lines. Moreover, we plan to map the global m5C profile in RNA of ICF1 patient-derived iPSCs and isogenic lines generated following correction of DNMT3B mutations, in order to understand whether DNMT3B dysfunction directly influences this specific modification at transcripts and more generally the biological significance of RNA methylation in a model mimicking the early stage of disease development.

Identification of novel proteins interacting with imprinting control regions in allele specific manner

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In mammals, several genes have been reported to be monoallelically expressed in parent of origin dependent manner. The expression of imprinted genes is under the influence of imprinting control regions (ICRs). During gametogenesis, differential DNA methylation marks are established at ICRs that binds crucial factors of genes expression. The sequential recruitment of various interacting partners, like RNA polymerases, transcription factors, and enzymes at ICRs govern monoallelic expression of imprinted genes. The protein ZFP57, binding the methylated allele at the ICRs, has been shown to be required for imprinting maintenance in early mouse embryo, recruiting the co-repressor KAP1, DNA methyltransferases (DNMT1,3A,3B) and the histone H3 lysine 9 methyltransferase SETDB1. ZFP57, thus play a role in monoallelic expression of a gene.

Our goal is to identify factors, which can be the possible partners of ZFP57, binding the methylated allele at ICRs, able to prevent the acquisition of DNA methylation, binding unmethylated allele, playing a role as a pioneer factors to open up the chromatin and initiate transcription or in maintaining the open chromatin state. To identify potential candidates and investigate their role, I have performed the chromatin-immunoprecipitation sequencing (ChIP-seq) analysis of datasets obtained from public repositories (NCBI-GEO) of mouse embryonic stem cells (mESCs). I further prioritized candidates at ICRs by visualizing peaks in UCSC genome browser. Using this approach, I identified nuclear respiratory factor 1 (NRF1) was binding at six ICRs in mESCs. We further confirms its allele-specific binding at unmethylated allele by ChIP in hybrid mESCs. Currently, we intend to find its binding in Zfp57-knockout hybrid mES cell lines. This approach has given us the opportunity to identify a new protein involved in regulation of genomic imprinting.

Session 2:

Structure and Functions of Biomolecules

In-situ single cell lipidomics by Imaging Mass Spectrometry

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Cell-to-cell variability is a fundamental feature of multicellular systems and a key aspect of cell sociology. In these contexts single-cell genomics, transcriptomics, metabolomics and proteomics (but not lipidomics) have helped elucidating the role of cell-to-cell heterogeneity in physiology and disease. Nevertheless, standard approaches fail to provide information on single-cell composition as well as on the spatial distribution of metabolites.
Over the past 20 years, mass spectrometry has shown noteworthy improvements thanks to the development of Imaging Mass Spectrometry (IMS) that enables the correlation between molecular information and spatial localization of the analytes. We have recently developed a protocol for in situ single-cell lipidomics based on MALDI-IMS and we were able to simultaneously image >100 individual metabolites in single-cells while in their context.
According to this procedure, primary human fibroblasts, were analyzed and the final distribution images of specific ions were generated. The quality of the mass images obtained was good enough to discriminate single cells as recognized by optical images. Then, analyzing the relative levels of > 250 m/z peaks, we found that compounds belonging to the glycosphingolipid class show a remarkably hyper-variability compared to other lipids. In particular, glycosphingolipid belonging to different series, ganglio- or globo-, showed a mutual exclusion in their expression at single cell level. We also developed an IMS-fluorescence imaging correlation method to have a more integrated approach to image cells. According to this procedure, fixed cells were stained with different fluorescent markers and images were acquired in a specific area by both confocal microscopy and IMS.All these data obtained till now represent a proof of principle for the feasibility and relevance of single-cell lipidomics/metabolomics.

Self-assembling proteins: GADD45γ amyloid-like cytotoxic aggregates form "*non-toxic*" hydrogels and nanogels

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The uncontrolled self-assembling of proteins frequently leads to pathological states. The activities carried out in the first year demonstrated that GADD45β, a member of the GADD45 family [1, 2], undergoes denaturation by forming β-rich amyloid-like aggregates that are cytotoxic in physiological conditions. The characterization of the unfolding process of the two other members of the family (GADD45α and GADD45γ) highlighted analogies and differences. Indeed, while GADD45α displays behaviour somehow similar to that exhibited by GADD45β, GADD45γ exhibits a partial and reversible unfolding without forming any aggregate. During the second year, the activities have been focussed (a) on the analysis of the determinants that favour/disfavour amyloid-like aggregation in GADD45β and (b) on the characterization of the GADD45β aggregates. Investigations carried on the first topic led to the identification of GADD45β variants with either increased or reduced propensities to form amyloid-like aggregates. Further studies, which include limited proteolysis experiments, are underway to identify the protein region(s) involved in the self-aggregation events. Notably, we found that GADD45β amyloid–like aggregates are able to form hydrogels and nanogels. The potential of these self-assembled systems, that have been characterized using a repertoire of structural/biophysical techniques, as innovative biomaterial is currently under investigation.

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Biochemical studies of PON2 and its variants: a protein strongly implicated in development of metabolic diseases

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The paraoxonases (PONs) i.e. PON1, PON2, and PON3 constitute a family of enzymes that have antiatherogenic and anti-inflammatory properties.
PON2 is an intracellular protein detected in mitochondria, endoplasmic reticulum and perinuclear region but it can translocate to the plasma membrane to counteract lipid peroxidation.
Although polymorphisms in the PON2 protein (i.e. S311C and A148G) have been associated with several metabolic diseases, its role in humans remains poorly understood.
Wild type and polymorphic variants of PON2 were characterized in E.coli and Sf9 cells to determine its 3D structure and biochemical functions, focusing on the role played by Post Translational Modifications (PTMs) in modulating PON2 catalytic activity.
In the *E.coli* expressed PON2 the polymorphic variants C311 and G148 were dramatically less active and PON2 resulted ubiquitinated in intact cells. Now we aim to understand the role of residues 311 and 148 in PON2 activities and if they are modulated by ubiquitinations. By a meta-analysis approach, based on coordinated control of gene expression through RNA-binding proteins to conserved short sequences at 3' or 5' UTR, we identified a human gene-cluster containing PON2 and 21 genes, all functionally related. Among them genes encoding for four E3 ubiquitin ligases and for two RNA binding proteins (RBPs) were found. The correlation between PON2 ubiquitination and these E3 ubiquitin ligases was investigated by gene silencing experiments and the effect of PON2 ubiquitination will be evaluated by mass spec analysis.

Structural analysis of the interaction of human prion protein with Cu2 + ion by nuclear magnetic resonance

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The cellular prion protein is a membrane glycoprotein abundantly expressed at the neuronal level. Following a conformational change, this protein converts to the pathological isoform scrapie, whose characteristics are different than the prion protein in physiological conditions. It has been shown that this transformation turns out to be a key event in the pathogenesis of prion diseases. My study shows the binding mechanisms of the human prion protein HuPrp90-231 and HuPrp23-230 to the copper ion, through nuclear magnetic resonance. The purpose of this work is to identify the amino acid residues of the prion protein involved in the copper bond and his possible role in the conformational change of this protein.
In conclusion, a stable interaction between the metal ion and the prion protein has been demonstrated, both in the full lenght form and in the 90-231 segment, at pH 5.5 and at a temperature of 298K. In particular, an interaction has been observed between the copper and the HuPrp90-231 near the His 96 and His 111 residues, while in the case of the full length form, near the four histidines present in the four octarepeat sequences (His 61-69-77-85).

UHPLC-ESI-qTOF-MS/MS metabolic profiling of food by-products with nutraceutical and cosmeceutical value

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The link between health and food was established long ago, but only in recent years nutrition and nourishment are re-proposed as an opportunity to prevent the most challenging health problems and manage the common ones. In this contest, the aim of the PhD project is to perform activities focused on the recovery of high added-value metabolites from food and its by-products for formulating new dietary and nutraceutical/cosmeceutical healthy products.
In particular, during the 2nd year of the PhD course, hempseed oil-like extracts, peculiarly enriched in PUFAs, were prepared to be used as dietary supplement, components of functional foodstuffs, and/or for cosmetic applications. Parameters such as extraction time, and solid-to-solvent ratio in ultrasonic extraction were evaluated to obtain the maximum yield without impairing sensory qualities and acceptability through the formation of lipoperoxidative secondary products. The metabolic profiling was achieved by ultrahigh-performance liquid chromatography (UHPLC) coupled with electrospray ionization (ESI) quadrupole/time-of-flight (QqTOF) mass spectrometer. The sensitivity and selectivity of the MS method allowed the identification of non-psychoactive phytocannabinoid acids differently oxygenated, never detected before in hempseed oils. The presence of these constituents could give additional nutraceutical value to the obtained extracts.

Exploring the molecular mechanisms of action of "alien metabolites"

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Invasive green algae of the genus *Caulerpa* are transforming vast Mediterranean areas in alarming monocultures. Besides the direct deleterious effect of their invasion, it is worth noting that their bioactive compounds can critically affect native species. Indeed, the accumulation of the algal bis-indolic alkaloid caulerpin (CAU) in the tissues of the native fish *Diplodus sargus* eating on *Caulerpa cylindracea* correlates with metabolic disorders in the fish. We clarified the molecular mechanisms by which CAU alters lipid metabolism in *D. sargus*. However, we are also investigating if other related physiological systems are affected. In particular, a further study aims to unravel if CAU is also able to induce significant changes in the endocannabinoid system functionality and/or affect the inflammatory response. Using the larvae of zebrafish (*Danio rerio*) as fish model we are assessing variations in lipid content using AdipoRed staining, while changes in endocannabinome and inflammatory response are investigated by LC-MS and gene expression analysis Finally, even though *C. taxifolia*, the so-called "killer alga", is also known to contain CAU, our chemical investigations led us to rule out the presence of the compound in *C. taxifolia* var. *distichophylla*, a strain recently found along the Sicilian coast.

Strategies based on biomaterials and stem cells for pulmonary tissue engineering

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Chronic lung diseases (CLD) are characterized by derangements of the alveolar walls and altered alveolar functions; they include severe diseases such as chronic obstructive pulmonary disorder and bronchopulmonary dysplasia of prematurity. A common denominator of these diseases is the palliative effect of current therapies and consequent absence of injury resolution, leading to distorted tissue repair. Mesenchymal stem cells have been demonstrated great potential about the regeneration of the lung injury in CLD, thanks to their ability to promote the alveolar development. However, the regeneration of the lung damage through only stem cells based therapies is more difficult to achieve as the loss of the supportive extracellular matrix that provides the lung scaffold, caused by these pathologies, may preclude repair of normal alveolar structure. This research activity aims to use polymeric biomaterial scaffolds combined with stem cells to allow pulmonary tissue regeneration for the treatment of the pulmonary damage in the CLD, using injectable polymeric biomaterials, that mimics the natural architecture of lung, based on polysaccharides such hyaluronic acid, and proteins such Collagen. Stem cells deriving from human umbilical cord have been used, for their ease of collection and very low immunogenicity, to promote lung regeneration synergistically with the biomaterials.

Structural and biochemical insights into p150 subunit of Chromatin Assembly Factor 1(CAF-1), a new tumor associated protein

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The Chromatin assembly factor 1 (CAF-1), formed by 3 subunits (p150-p60-p48), is a histone chaperone responsible for the positioning of histones into nucleosomes on newly synthesized chromatin. The over-expression of p150 subunit has been associated with neoplastic progression of many human malignancies, indicating an important role of this subunit in cancer; however, the molecular mechanism through which this occurs remains unknown. In addition, structural information about p150 is still missing. Trying to fill this gap, the aim of my phD is to provide insights into the biochemical and structural features of p150 subunit. Based on biochemical and bioinformatics approaches, our results show that the domain containing the amino acids ranging from 880 to 956, successfully expressed in *E.coli*, purified and characterized, belongs to the family of Intrinsically Disordered Proteins (IDPs). Despite being globally unfolded, this domain seems to possess some polyproline II – (PPII) secondary structure and acquire some elements of secondary α-helical structure under experimental conditions. The observed flexibility of this domain may have several roles in tumor progression. The structured elements within this domain might be involved in mediating interactions with p150 partners. Considering the known role of IDPs in diseases, this result could be the first highlight on the possible involvement of IDPs in p150 tumor progression.

Chimeric Recombinant Antibody Fragment of anti-Nodal 3D1 and anti-Cripto 1B4 for theranostic applications

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Nodal is a potent embryonic morphogen belonging to the TGF-beta superfamily. Typically, it binds to the Alk4/ActRIIB receptor complex in the presence of the co-receptor Cripto-1. Nodal and Cripto-1 expression is restricted to embryonic tissues and human embryonic stem cells, whereas it is absent in normal adult cells [1]. Re-expression of the proteins in the adults is associated with a large number of tumors where controls intracellular signaling and promotes tumorigenesis [1]. The two proteins have been thereby indicated as a diagnostic biomarkers and therapeutic targets for several types of cancer. We have generated anti-Nodal and anti-Cripto-1 monoclonal antibodies named 3D1 and 1B4, respectively. 3D1 therapeutic efficacy has been proven in aggressive melanoma both *in vitro* and *in vivo* models [2,3,4]. We have also generated partly humanized recombinant Fabs of 3D1 and 1B4 to obtain new molecules with better PK/PD profiles [5]. Here, we present the biochemical characterization of the 3D1 recombinant chimeric Fab efficiently produced in the *E. Coli* host using the periplasmic expression strategy. This chimeric Fab fragment will be employed as scaffold to generate new properly engineered Nodal targeted-theranostic agents [6] and bispecific anti-Cripto-1/anti-Nodal Fab2 for both imaging and therapeutic purposes in Cripto-1/Nodal positive tumors.

Ribotoxin-like proteins in edible mushrooms: purification, characterization and their possible cytotoxic activities

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Ribotoxins are a group of fungal extracellular rRNA ribonucleases that catalyse the hydrolysis of the phosphodiester bond between G4325 and A4326 in rat 28S rRNA causing the inhibition of translation (1). The biological role assigned to ribotoxins is due to insecticidal properties, supporting their involvement in defence and parasitism. On the other hand, extensive research has been conducted to investigate their use as antitumor agent. Ribotoxins are found in Ascomycota phylum and only more recently a new member has been isolated from the Basidiomycota phylum suggesting that ribotoxins are more widely distributed among fungi than previously reported (2). This novel ribotoxin, named Ageritin purified from *Agrocybe aegerita* (V. Brig.) Singer shows peculiar structural and enzymatic features and, interestingly, displays cytotoxic activity towards several tumour CNS model cell lines (2). The discovery of Ageritin open new scenarios in the research of ribotoxin-like proteins from Basidiomycota phylum.
Therefore, during the first PhD year, I have performed a screening of other mushrooms belonging to Basidiomycota phylum, by using a standard protocol for purification of basic proteins, and verifying the presence of ribonucleolytic activity in fruiting bodies. Furthermore, the isolation of these ribotoxin-like proteins will be extended to mycelium of them.

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Untargeted Metabolomics evaluation of nutraceuticals using NMR as main analytical platform

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The importance of functional foods, nutraceuticals and other natural health products has been well recognized in connection with health promotion, disease risk reduction and reduction in health care costs. Metabolomics is a powerful approach because metabolites and their concentrations, unlike other "omics" measures, directly reflect the underlying biochemical activity and state of cells / tissues. In this project we try to focus on the study of metabolomics profiles of potentially nutraceutical products. Different cultivar of *Prunus persica* have been analyzed through an NMR metabolomics approach which, thanks to high reproducibility of technique, allows the quantification of the concentration and the study of the chemical structure of metabolites. At this stage the research have been mainly focalized on the whole phytochemical part. Considering the prevalent extractive system utilized, that is able to mainly extract primary and secondary polar metabolites using an hydroalcoholic solution, we proceeded to set up and standardize a micro extractive biphasic system using $CDCl_3/CD_3OD/D_2O$ in order to extract lipophilic secondary metabolites too. Additional activities in the research have been to test the *radical scavenging* of the extracts with various in vitro assays. Moreover, to define the structure of the metabolites responsible for the registered activities, an examination of more promising extracts have been done using 2D-NMR techniques.

Structural and functional insights into complement modulation by the complement regulatory protein CD55

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The complement system is an intricate network of plasma and membrane-associated proteins involved in adaptative and innate immune responses. Due to its toxic functions, the complement system is tightly regulated by complement regulatory proteins. Of these, CD55 plays a central role by inhibiting the formation of the C3 convertases and has been linked to several diseases including cancer, malaria and multiple sclerosis. CD55 overexpression is strongly correlated to resistance to immune treatments in cancer. Also, it has been shown that hampering the complement inhibiting activity of CD55 improves immune therapies by enabling selective cancer cell killing by the complement system.
In this study, we aim at the definition of the structural features responsible for CD55-mediated inactivation of the complement system. We successfully expressed and purified CD55 and designed a panel of molecules potentially able to bind CD55 and hamper the inhibition of the complement system. Currently, we are performing structural and binding studies on these complement modulators to optimize them via rational molecular design. Besides exploring the structural determinants of complement modulation by CD55, our study aims at the development of novel molecules with modulating activity of the complement system, for applications in the diagnosis and treatment of diseases associated with CD55 overexpression.

Development of inhibitors targeting AIF/CypA lethal complex

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The complex formation between AIF and CypA proteins, following oxidative stress in neuronal cells, provides cell death by a caspase-indipendent mechanism.[1,2] AIF interacts with CypA, mainly through the β-hairpin region spanning residues 370-394.[3] A synthetic peptide covering this region, hereafter AIF(370-394), inhibits the complex formation *in vitro* and provides neuroprotection in cell lines, treated with glutamate, by competing with AIF for the same interaction site on CypA.[4,5] Data obtained demonstrated that the AIF(370-394) is a good model for studying the AIF/CypA complex and for developing new inhibitors with potential therapeutic value.
First, to improve the biological activity of the AIF(370-394) peptide, we have designed and synthesized AIF constrained peptide analogues, to limit the molecule conformational freedom and to induce a more native-like conformation.
Then, we have investigated the conformational features of a series of bi- and mono-cyclic analogues containing both disulphide and 1,4-disubstituted 1,2,3-triazole bridges.[6]
Biochemical assays have been also developed to evaluate the ability of analogues to bind CypA[3-5] and to obtain structure-activity relationship insights. Results showed that the introduction of two bridges, significantly enhances both the secondary structure content and the target affinity. Data obtained offer the structural bases for the design of new inhibitors of AIF/CypA complex.

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Mechanism of BARS-mediated mitotic Golgi fragmentation

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One of the key mechanisms that control the cell cycle progression is the "Golgi mitotic checkpoint". During cell division, the Golgi complex undergoes extensive fragmentation to allow its correct partitioning into the daughter cells. The inhibition of this fragmentation results in a G2-phase block. We have identified CtBP1-S/BARS as key controller of the mitotic Golgi ribbon unlinking.
To define this CtBP1-S/BARS-mediated fission processes we have identified the CtBP1-S/BARS complex components. This complex comprises CtBP1-S/BARS bridged to PI4KIIIβ through dimeric 14-3-3γ, ARF, PLD1/2 and the two stabilizing kinases PKD and PAK, which phosphorylate PI4KIIIβ and CtBP1-S/BARS, respectively. Once incorporated into this complex, CtBP1-S/BARS binds to, and activate two Golgi resident lysophosphatidic acid acyltransferase enzymes (LPAATs), namely LPAATγ and LPPATδ.
We are currently investigating the role of each protein-complex component in cell-cycle synchronized HeLa cells. The specific depletion/inhibition of CtBP1-S/BARS, LPAATγ, LPAATδ, PAK1/2 or PLD1/2 strongly inhibits the mitotic Golgi fragmentation (although to different extents) underling the role of these CtBP1-S/BARS complex components in the mitotic Golgi partitioning.
The role of the catalytic activity of LPAATγ and LPAATδ, once incorporated into the CtBP1-S/BARS complex, is under investigation.

Secondary metabolites from Mediterranean plants for nutraceutical and pharmaceutical applications

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Secondary metabolites (SM) represent an exciting library of bioactive compounds, which can be used as nutraceuticals and pharmaceuticals. Nowadays, the challenge for pharmacology is to describe and understand the diversity of SM, their modes of action alone or in natural combinations as found in plants (1).
Thus, a screening of four plants (*Myrtus communis*, *Plagius flosculosus*, *Helichrysum saxatile* and *Scrophularia trifoliata*) is the aim of this first year of PhD course. This work is heavily supported by Nuclear Magnetic Resonance (NMR, 1D and 2D) that provide an overview of the metabolomes and allowed to elucidate the structure of the molecules potentially responsible for the activities. The ^{1}H-NMR analysis shows a different composition in secondary metabolites, especially for species belonging to different families. Feruloylquinic acids, acylphloroglucinols, iridoids and flavonoids are the main identified SM. MTT assay (2) was performed in order to evaluate the extract's anti-proliferative activity to HepG2 and HuH-7 liver cell lines. Subsequently, other tests also assessed their potential antimicrobial (3) and anti-HIV. From these essays, the most promising results derive from *M. communis*, while the other plants respond differently according to the assay carried out. Considering the results, a survey on bioactive fractions and single compounds will be useful to understand the metabolites responsible for the bioactivity and their mode of action.

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Biological activities of the ribotoxin Ageritin from *Agrocybe aegerita*: a possible novel neurotoxin as tool to study nervous system model cells

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Ribotoxins are a group of specific extracellular rRNA endoribonucleases produced by Ascomycota phylum that display cytotoxicity towards animal cells, having been proposed as both insecticidal and antitumor agents. Moreover, they could also be used as specific tools for the study of human ribosomopathies (1).
During the first PhD year, a ribotoxin (Ageritin) has been isolated from *Agrocybe aegerita* belonging to Basidiomycota phylum suggesting that ribotoxins are more widely distributed among fungi than previously believed (2). In order to gain insights into the biotechnological and biomedical applications of Ageritin, we have found that Ageritin: (i) displayed rRNA endonuclease activity against microbial ribosomes; (ii) was active against the Tobacco mosaic virus RNA; and (iii) displayed endonuclease activity against a supercoiled plasmid. On the other hand, for the first time we have found that ribotoxins such as ✔-sarcin and Ageritin displayed antifungal activity against the green mold *Penicilium digitatum* and their mechanism of action was also investigated (3).
Finally, given its cytotoxic activity against SK-N-BE(2)-C, U-251 and C6 cell lines, Ageritin was tested towards SH-SY5Y human neuroblastoma cell line, confirming its cytotoxicity by promoting apoptosis and candidating it as novel neurotoxin and tool to study nervous system model cells.

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Effects of Zn(II) replacement with Pb(II), Hg(II) or Ni(II) on the structure and function of prokaryotic zinc-finger domain

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Zinc ion binding to a proteic domain is a principal event in the achievement of the correct fold in the classical zinc finger domain since the motif is largely unfolded in the absence of the metal. In the case of prokaryotic zinc finger the bigger 🚲🚲🚲✔✔ domain contributes to the folding mechanism also with a larger hydrophobic core. For these reasons, following the great attention devoted to unveil the effect of a xenobiotic metal ion replacement in zinc fingers and in zinc-containing proteins in general, the prokaryotic zinc finger domain appears to be an interesting model to study metal ion interaction with metallo-proteins.
Here, we explore the binding of Ni(II), Hg(II) and Pb(II) to Ros87, the DNA binding domain of the prokaryotic zinc finger protein Ros. We measured the Ros87-metal ion dissociation constants, and monitored their effect on the folding and function of the domain. Interestingly, we found that the nickel ion is capable to fold the protein, while in presence of lead and mercury Ros87 does not appear correctly folded. Accordingly, an overall strong reduction in DNA binding capability is observed for Pb(II) and Hg(II). Our data integrate and complement the information collected in the last few years about the functional and structural effects of metal ion substitution in classical zinc finger in order to contribute to a better comprehension of the well-known toxicity of these metals in biological systems.

Session 3:

Molecular Cell Biology

Obesity-Driven Neurodegenerative diseases: new insights for new molecular interplayers and therapeutic targets

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Neurodegenerative diseases are one of the main causes of worldwide disability and decreased quality of life and are increasingly linked with obesity. The available treatment strategies against both pathological conditions remain ineffective. Dysmetabolic and neurodegenerative diseases share some molecular pathways in several brain areas, starting from the hypothalamus, the most extensively interconnected area of the brain accordingly to its inherent functional activities. In this area we studied the mechanisms that control the balance between the phosphorylated and not phosphorylated form of Tau, a protein that modulates the stability of axonal microtubules and is implicated in synaptic plasticity. In the hypothalamus, we found different molecular interplayers altered in obese and neurodegenerative phenotypes, such as leptin hormone, orexin-A (OX-A) neuropeptide and the endocannabinoid system (ECs). All of them interact affecting the GSK-3β activity, which is responsible for the phosphorylation of Tau. Our findings showed that leptin deficiency leads to an increase of OX-A-induced biosynthesis of the endocannabinoid 2-arachidonoyglycerol (2-AG) paired with a sharp increase of pTau/Tau ratio. This condition was reverted after leptin treatment. On this basis, we hypothesized a functional orexin-endocannabinoid-leptin interaction as an upstream signaling pathway for the regulation of Tau phosphorylation. Another outcome reinforcing the important role of Tau in obesity-driven neurodegenerative diseases, was the difference of distribution and quantity of phosphorylated tau form in hippocampus and cortex of obese mice. Unraveling the functional cross-talk between ECs and OX system in the regulation of Tau phosphorylation in those areas involved in cognitive function could reveal novel molecular targets and pathways for novel therapeutic approach.

Regulation of PPARγ signaling through alternative splicing and dominant negative isoforms

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The increase of pro-inflammatory M1 macrophages in the adipose tissue is hallmark of hypertrophic obesity and correlates with low PPARγ activity and insulin resistance onset. PPARγ is the master transcription factor of adipogenesis. Accordingly, PPARγ mutated receptors with dominant negative activity have been associated with insulin resistance, increased T2D risk and defective adipogenesis. During my PhD, I have studied PPARγΔ5, a new not – functional dominant negative PPARγ isoform generated by alternative splicing - identified in our laboratory - physiologically expressed during adipocyte differentiation. My results indicate that ligand-mediated PPARγ activation is necessary to promote PPARG pre-mRNA splicing through the contribution of the splicing factor ASF/SF2. Interestingly, PPARGΔ5 is highly expressed in the adipose tissue of obese patients with type 2 diabetes and positively correlates with BMI. Adipose precursor cells isolated from SAT of obese patients expressing high levels of PPARGΔ5 have a reduced adipogenic capability. Accordingly, PPARGΔ5 over-expression in human mesenchymal stem cells (MSCs) dramatically impairs their adipogenic capability. Notably, human hypertrophic adipocytes, generated in vitro from MSCs using ad hoc protocol, showed an increased ratio of PPARGΔ5/PPARG vs mature ones. More interestingly, undifferentiated MSCs grown in conditioned medium of human M1 macrophages have significantly higher PPARGΔ5/PPARG ratio, suggesting a role for secreted soluble factors. Accordingly, treating undifferentiated MSCs with human recombinant cytokines (TNFα, IL1β and IL8) increases PPARGΔ5/PPARG ratio, similarly to hypertrophic adipocytes. These results suggest that inflammatory milieu that is generated in the hypertrophic adipose tissue may modify the relative expression of canonical and dominant negative PPARγ isoforms compromising ratio differentiation and insulin-sensitivity of adipose cells.

Mitochondrial dynamics as a new therapeutic target for neurodegenerative diseases

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Mitochondrial functions, such as respiratory function, biogenesis, trafficking, fission, fusion or mitophagy, are essential for the cells' well-being. Indeed, considerable evidence implicate mitochondrial dysfunction in the pathogenesis of a number of progressive neurodegenerative diseases, like Alzheimer's Disease, Parkinson's Disease and Down Syndrome (DS). Notably, in respect of an imbalance in mitochondrial dynamics, the emergence of a pathological phenotype -and especially of neurodegeneration- is inevitable as we age. Previous results from our lab show that targeting genes of the mitochondrial fusion/fission machinery can improve the mitochondrial dysfunction detected in human fetal-derived primary cells of DS. Aiming to identify and understand the impact of mitochondrial dysfunction in multiple neurodegenerative disease states, I am currently developing an unsurpassed assessment of its evolution in the aforementioned in vitro cell-based model of DS. By staining mitochondria, and by implementing confocal microscopy and sophisticated image processing software we are able to analyze their morphology in a quantitative manner, in order to assess a clear correlation between mitochondrial morphology and function. As the study is currently on-going, preliminary results will be presented. It is anticipated that a detailed understanding of mitochondrial dysfunction will enable the discovery of novel targets for therapeutic intervention within the neurodegeneration field.

Molecular imaging of vulnerable atherosclerotic plaque in murine model using high frequency & contrast enhanced ultrasound

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Background: Atherosclerotic plaques often develop asymptomatically until rupture, triggering acute myocardial infarction or stroke. Therefore, identify unstable plaques is a major clinical goal. Animal models of atherosclerosis are essential in understanding the mechanisms inducing plaque progression toward vulnerability.

$ApoE^{-/-}Fbn1^{C1039G+/-}$ mice are currently the experimental system that develop lesions more strictly resembling human vulnerable plaques, including neoangiogenesis, hemorrhages, spontaneous ruptures and sudden death for acute complications. These genetic mutations result in fragmentation of vessel wall elastic fibres, increased arterial stiffness and, in association with dyslipidemia and hypercholesterolemic diet, lead to highly unstable plaques.

Accordingly, they represent the most relevant tool to study pathophysiology of vulnerable plaque, and to investigate new strategies of diagnosis, risk stratification, or treatment.

Aims & **Methods**: This study aims to noninvasively and longitudinally characterize $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice by state-of-the-art ultrasound imaging, providing detailed morpho-functional and molecular information about their cardiovascular, renal and hepatic phenotype, complementary to body composition and biochemical parameters, and with histology and immunohistochemistry validation. Results will be compared to control C57BL/6J mice and $ApoE^{-/-}$ mouse model of stable atherosclerotic plaque, under normal or western type diet.

Conclusions: This study would improve translational knowledge for vulnerable atherosclerotic plaque risk assessment and prevention.

Keywords: vulnerable atherosclerotic plaque; phenotyping of preclinical models; high frequency & contrast enhanced molecular ultrasound

The saturation degree of fatty acids and their derived acylcarnitines determines the direct effect of metabolically active thyroid hormones on insulin sensitivity in skeletal muscle cells

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3,5,3' – triiodo – L – thyronine (T3) and 3,5 – diiodo – L - thyronine (T2) are both metabolically active thyroid hormones. Here their metabolic effect on rat skeletal muscle cells incubated with fatty acids was studied. In presence of insulin, the response to insulin in presence of fatty acids with a varying degree of saturation was inverted by both T3 and T2. The intracellular level of acylcarnitines was reduced by both hormones. Particularly, in the myotubes insulin resistance was increased by treatment with palmitoyl carnitine, while was reduced by treatment with oleyl- and linoleylcarnitines. Palmitate decreased cellular respiration that was normalized only by T3, through ATP synthesis-independent. T2 and T3 differentially regulated the expression of relevant genes involved in mitochondrial fatty acid uptake, fatty acid oxidation, glucose metabolism and insulin sensitivity in presence of each fatty acid. The results show that T2 and T3 both invert the fatty acid-induced response to insulin but through different mechanisms, and that the outcome depends on the degree of saturation of the fatty acids and their derived acylcarnitines.

Molecular pathways activated by Excitatory Amino Acids in spermatogenesis

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Spermatogenesis is a complex multi-phase process of continuous proliferation, differentiation and apoptosis of germ cells. Male fertility depends on the continuous daily production of billions of spermatozoa. The reduction of sperm concentration and motility constitutes the main cause of male infertility. The Project aims to study the molecular mechanisms evocated by amino acids currently in use as nutritional supplement to improve the spermatogenesis in infertile subjects and to discover if new excitatory amino acids (EAAs) can have the same effect. Particularly, it will be investigated the role of EAAs in the regulation/progression of spermatogenesis through their binding with ionotropic and metabotropic receptors (GluRs). In vivo experiments on Rattus norvegicus and in vitro experiments on germ and somatic cell lines will be carried out. It will be investigated the effects of the EAA/GluR signalling in: 1) mitotic and meiotic pathways; 2) apoptotic/autophagic pathways; 3) transduction of androgenic stimulus by Sertoli cells to germ cells. Further it will be considered the effects of EAAs on testis mitochondrial compartment, which has a pivot role in regulation of germ and somatic cell homeostasis. This study could have relevant implications in the field of male infertility.

Use of gene therapy for treatment of retina inherited dominant disorders

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At present therapies for inherited dominant disorders are not available, thus representing an urgent unmet medical and social need. Our group want to discover that transcriptional repression with synthetic transcription factors (TFs) embodies a novel therapeutically effective mode to treat the toxic effects of gain-of-function mutations causing incurable inherited dominant disorders (Botta S et al. eLife. 2016 Mar 14;5. pii:e12242. doi: 10.7554/eLife.12242). Mutations in the RHODOPSIN gene can cause the blindness disorder, autosomal dominant retinitis pigmentosa (ADRP). Our group demonstrated safety and efficiency of RHODOPSIN gene transcriptional silencing in pre-clinical animal models by a specific synthetic transcriptional repressor (ZF6-DB) delivered to the retina by an adeno-associated virus (AAV) vector (AAV-ZF6-DB). Notably, ZF6-DB blocks RHODOPSIN transcriptional activity without transcription repressor domains, which canonically in eukaryotes recruits co-repressor proteins. Thus, the "naked" ZF6-DB avoids protein-protein interactions limiting side effects. Furthermore, we showed that a single AAV vector containing two independent expression cassettes (AAV-ZF6-DB-hRHO), enables balanced silencing of RHODOPSIN by ZF6-DB and its simultaneous replacement with a human wild-type copy of the RHODOPSIN gene. Thus, supporting a novel therapeutic paradigm for treatment of genetic disorders caused by gain-of-function mutations. Based on these data we obtained the orphan drug designation (ODD) from the European Medicines Agency (EMA) and filed two patients, which protect this innovative therapeutic strategy. The objective of the inSight ERC-PoC grant is to support AAV-ZF6-DB-hRHO clinical translation, with the final goal of bringing this therapeutic to patients through market authorization and commercialisation.

Cripto modulates angiogenesis and EndMT by controlling the shaping of pro-healing macrophages in skeletal muscle regeneration

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Skeletal muscle regeneration requires the contribution of inflammation, which is driven first by pro-inflammatory and then by pro-healing macrophages. The mechanisms controlling these macrophage types are still poorly understood.

Here we show a novel role of the developmental factor Cripto, which is progressively expressed in a subset of pro-healing macrophages upon cardiotoxin (CTX) -induced acute injury. To address this issue, we obtained and analysed a myeloid lineage-specific Cripto loss-of-function mouse model with lineage tracing (Tg:Lyz2Cre::R26mTmG::Criptofl/fl; CriptoMy-LOF). According to Cripto expression profile, we found an impaired accumulation of pro-healing macrophages in CriptoMy-LOF mice upon acute injury. Of note, as recently emerged, pro-healing macrophages restrict the Endothelial-to-Mesenchymal Transition (EndMT) of endothelial progenitors. In line with this idea, CriptoMy-LOF injured muscle showed defective angiogenesis and increased EndMT. Despite this phenotype, regeneration was not significantly affected until after the second round of CTX-induced injury, when a significant decrease of the cross-sectional area was observed in CriptoMy-LOF muscles. To investigate this phenotype further, we evaluated the impact of CriptoMy-LOF in the mdx model of Duchenne muscular dystrophy. Strikingly, we found the loss of Cripto in macrophages induce the worsening of the disease.

Collectively, our findings shed light on a novel role of Cripto in modulating the inflammatory response both in acute injury and disease, with a consequent impact on angiogenesis and muscle regeneration.

In vivo evaluation of ellagic acid and curcumin effects in *Danio rerio* embryos

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Oxidative stress can cause structural and functional alterations of DNA during delicate life phases as embryonic development. Specific antioxidants could protect organisms from ROS attack, in particular our previous studies showed an antigenotoxic potential of curcumin (CUR) and ellagic acid (EA) on human amniotic cells. In order to evaluate long-term effects on embryo development, Danio rerio embryos was exposed to different concentration of CUR, EA and a genotoxic agent (H2O2), alone and in combination. Results showed morphological alterations induct by H2O2 treatment, such as body hypopigmentation, calf sac edema and altered natal movements. None of these alterations were found after EA treatment, moreover cytotoxicity and genotoxicity tests (DCF Assay and RAPD-PCR (GTS%)) confirmed that the EA exposure did not induce damage to any biological levels, except for the highest concentration tested. While, significantly mortality after CUR exposure was observed. Interesting results were highlighted following the co-exposure to EA 5 mM + H2O2. In fact we observed an increase in embryos survival, a reduction of % intracellular ROS, and an increase in the GTS% respect to H2O2 single treatment. So EA could be considered as a powerful antigenotoxic agent, able to protect the earliest stages of ontogenesis from mutagenic substances.

Role of Glycosphingolipid metabolic reprogramming in Neuronal Differentiation

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Glycosphingolipids (GSLs) are a heterogenous group of amphipathic lipids composed of a hydrophobic ceramide base and a sugar headgroup. The ceramide is integrated in the cellular membranes (mostly plasma membrane) whereas the sugar headgroup is seen facing the non-cytosolic space. Based on the kind of the glycosylation, these lipids can be classified into 4 major groups, namely; Globosides, Gangliosides, Lactosides and Asialosides. This diversification is regulated via the multitudes of glycosphingolipid synthesizing enzymes and key adaptors that take part in the sequential glycosylation cycles and transport of these lipids. Over the course of neuronal differentiation, Embryonic Stem cells, predominantly expressing globosides, gradually switch to expressing gangliosides exclusively. Previous published data (Russo et al., 2018) from the lab exhibited that this switch was internally regulated by the GSLs themselves and that by upsetting this switch (by feeding cells exogenous globosides), one could block neuronal differentiation and retain 'stemness' markers (OCT4, NANOG etc.). Data from other groups (Liang et al., 2010) suggested that this switch is transcriptionally regulated. To study this switch chronically, we made stable mouse E14 embryonic stem cell lines overexpressing human Gb3S, which is the synthesizing enzyme of the first globoside (Gb3) in the sequential glycosylation process, under a constitutively active chicken β-actin promotor. The aim was to build upon the previous data published from the lab which showed that the lipid Gb3 negatively regulates the expression of neuronal protein AUTS2, thereby, blocking neuronal differentiation but, what we discovered was that the Gb3S was being actively degraded only upon differentiation whereas the protein was stable in the stem cell state, thereby bypassing the differentiation 'block'. This hints towards the possibility of another layer of post-transcriptional regulation of the Gb3S enzyme further cementing the hypothesis that this switch is extremely important for proper differentiation and development.

Novel fluorescent probes for precision labeling in super-resolution microscopy

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The development of novel bioconjugation strategies is becoming a very broad field of research,which has a strong impact on many fields related to life science, such as theranostics and advanced imaging. My project is focused on the development of novel staining reagents for super resolution imaging. Currently, a major problem in super resolution imaging systems is related to the precision of labeling the protein target of interest. Indeed, there is a problem common to every light microscopy technique related to the use of conventional markers for cellular staining. With antibody probes and classical immunofluorescence protocols, there is always a localization uncertainty of 20-40 nm because the emitters are far from the target of interest. Our research is focused on the development of novel Fab-based staining reagents for super resolution imaging that can be able to break this localization uncertainty by a precision labeling. To accomplish this, we are setting up an N-terminal selective reaction to ensure that the fluorescence emitters located there are very close to the epitope. The aim of my research project is the characterization of the selective N-terminal labeling reaction of Fab by HPLC and Mass spectrometry analysis.

sPLA2-IIA regulates osteoclast differentiation and function

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Secreted phospholipase A2 group IIA (sPLA2-IIA) is involved in osteoclastogenesis, which leads to the formation of multinucleated professional bone-resorbing osteoclasts. To deepen the role of this glycerophospholipid-hydrolytic enzyme, the Raw264.7 macrophagic cell line was chosen as osteoclast precursors, which can be differentiated through 4-5 days of treatment with Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL). As sPLA2-IIA is a dual-function protein, inhibitors with distinct selectivity against sPLA2-IIA actions were used, to dissect out its mechanism(s). During RANKL-induced osteoclastogenesis of Raw264.7 cells, a highly hydrophobic pentapeptide [c(2NapA)LS(2NapA)R; 20 μM] and a cell-permeable small molecule [KH064; 40 μM], decreased transcription of osteoclast markers and multinucleated cell formation. Down-regulation of sPLA2-IIA expression, using small-interfering-RNAs in the precursor cells, confirmed these data. Instead, treatment with an alkylating reagent of the catalytic histidine of sPLA2-IIA [p-bromophenacyl bromide; 10 nM] reduced osteoclast maturation without blocking syncytium formation. These data indicate that sPLA2-IIA participates in osteoclast maturation and control of syncytium formation by mechanisms that may be both catalytically dependent and catalytically independent via interactions with an unidentified partner. Data obtained with addition of both wild-type and catalytically inactive recombinant sPLA2-IIA throughout the differentiation of Raw264.7 cells reinforces this interpretation. Further support comes from primary osteoclast precursors isolated from sPLA2-IIA knock-out BALB/cJ mice. Indeed, RANKL-induced differentiation of sPLA2-IIA knock-out precursors generated less TRAP-positive osteoclasts, with lower transcription of osteoclast markers and bone resorbing activity, compared to wild-type controls. Of note, osteoclast-differentiated Raw264.7 cells showed increased activation of p-38 SAPK, while c(2NapA)LS(2NapA)R and KH064 treatments regulated RANKL-induced signalling through a decrease of p-38 activation. Moreover, treatment with a p-38 inhibitor [SB203580; 25 μM] impaired formation of multinucleated cells without affecting osteoclast maturation, which indicates the involvement of p-38 signalling downstream of sPLA2-IIA in osteoclast fusion. The further definition of the sPLA2-IIA determinants in osteoclast fusion will provide us with a more complete understanding of the still enigmatic osteoclastogenic process.

Targeting the cancer (stem) cells – tumor microenvironment crosstalk to improve pancreatic cancer prognosis

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To date, most efforts to identify new therapeutic targets for pancreatic cancer (PC) have focused on tumor cells themselves or on the tumor as a whole. Histologically, however, PC consists of a heterogeneous population of tumor cells and desmoplastic stromal tissue, which account for up to 90% of the tumor area. The formation of distant metastases is the deadliest phase of cancer progression and the stromatic compartment plays a critical role during this process. Current molecular diagnosis of PC is exclusively based on the analysis of epithelial cells not considering the stromatic compartment. Interestingly, a broad subset of PCs is characterized by high levels of TGF-beta superfamily ligands such as Nodal, Activin and TGFB1 (N-A-T) and by the prominent TGFB signaling in tumor stromal cells in the primary tumors and in the metastatic site. The main goal of this project is to unravel how the microenvironment of the primary tumor helps tumor cells to colonize a distant organ. Specifically, we will: 1) Obtain pure subpopulations from epithelium and stroma of PC human samples; 2) Define the role of N-A-T during the tumor-stroma crosstalk; and 3) Determine whether the metastatic cells activate the N-A-T -driven stromal response at the metastatic site.

Unravelling autoregulatory signalling circuits controlling export of different cargo classes from the ER

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Biosynthetic membrane transport involves the synthesis, folding, processing and sorting of proteins and lipids across anatomically separated compartments from the Endoplasmic Reticulum (ER) to the Golgi and then the endolysosomes. Communication between compartments is predominantly mediated by large fluxes of membranous vesicles of various shapes and sizes. Membrane transport hence is subject to deviations from equilibrium under physiological and pathological stresses that have to be maintained to preserve optimal function and homeostasis of the transport apparatus. Autoregulatory devices, variably called control systems, comprise of sensor, controller and effector mechanisms to maintain optimal function and homeostasis of key steps in membrane transport via signalling or transcriptional cascades. We have previously identified and characterized such a mechanism at the ER that senses folded cargo loads and prevents potentially dangerous cargo accumulation. Using temperature sensitive cargo proteins ts045 VSVG and Procollagen I, we observed that cargo folding and binding to specific isoforms of COP-II component sec24 induces the assembly and activation of a multicomponent Gα12-PKA dependent signalling cascade at the ER exit sites (ERES) called AREX (autoregulation of ER export) that accelerates cargo export to the Golgi. This mechanism however is not generally applicable to all cargos exiting the ER, as soluble secretory human growth hormone (hGH) is not under control of the canonical AREX cascade. We report in this study that hGH occupies and utilizes a pool of ERES that are distinct from AREX dependent cargo VSVG. Moreover, we suggest that different cargo classes exiting the ER activate distinct autoregulatory signalling cascades that coordinate the process of cargo synthesis, folding and export to the Golgi.

A novel ultraconserved element containing long noncoding RNA is required to preserve transcriptional dynamics and maintain embryonic stem cell selfrenewal

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Ultraconserved elements (UCEs) show the peculiar feature to retain extended perfect sequence identity between human, mouse, and rat genomes. Most of them are also transcribed and identify a new family of lncRNAs, the transcribed UCEs (T-UCEs). Despite their involvement in human cancer, their physiological function is largely unknown particularly in stem cells. We identify a lncRNA containing the uc.170+, named T-UCstem1, and we highlighted a functional interplay between it and the neurogenic miR-9. Such molecular interplay is crucial in preserving the ESC proliferation; indeed, T-UCstem1 Knock-down embryonic stem cells ((T-UCstem1 KD ESCs) showed an increased mir-9 levels leading to a cell cycle perturbation. Further analysis showed that a T-UCstem1/miR-9/Lin28b axis controls cell cycle progression in ESCs. Moreover, we showed that, despite their altered proliferation rate, T-UCstem1 KD ESCs retained a proper pluripotency in vitro and in vivo. Additionally, we compared RNA-seq profiling of T-UCstem1 KD and Control ESCs. The analysis showed a large number of the gene up-regulated in T-UCstem1 KD ESCs and most of them are bivalent domains-associated genes. Such observation led us to hypothesize and prove the involvement of T-UCstem1 in preserving the epigenetic status of such regulative elements, by stabilizing Polycomb Repressive Complex 2. All together, our findings provide unprecedented evidence that T-UCEs regulate physiological cellular functions and point to an essential role of T-UCstem1 in preserving ESC identity.

Mechanism of Interaction of Glycerophosphoinositol and Shp-1

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Glycerophosphoinositols are biologically active metabolites produced from membrane phosphoinositides by phospholipase A2IValpha. When added exogenously, the glycerophosphoinositols can enter cells and have multiple effects. Previous studies conducted in our lab have unveiled that glycerophosphoinositols impair tumor cell migration through the extracellular matrix by melanoma cells, whereas glycerophosphoinositols act as paracrine factors in a negative feed-back loop that decreases pro-inflammatory response in LPS-treated human monocytes affecting the expression of key pro-inflammatory mediators. Currently, we are focusing our attention on the ability of these metabolites to induce remodeling of the actin cytoskeleton. With the aim to elucidate the underlying mechanism of action of glycerophosphoinositols, our lab has found that the tyrosine phosphatase Shp1 acts as a specific intracellular receptor of the glycerophosphoinositols. In order to ascertain the domain involved in the regulation of the phosphatase activity and in the binding of glycerophosphoinositols to Shp1, we performed various biophysical methods, NMR spectroscopy, mutagenesis and structural analysis studies, resolving the domain of Shp1 involved in this binding. The full definition of the specific protein domain/s involved in the binding of the glycerophosphoinositols will allow the full definition of the mechanism of action of these compounds.

Mutual suppression of miR-125a and Lin28b in human hepatocellular carcinoma cells

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MicroRNA-125a-5p (miR-125a) is a vertebrate homolog of lin-4, the first discovered microRNA, and plays a fundamental role in downregulating Lin-28 protein. Lin-28 is considered a gatekeeper molecule that regulates the transition between pluripotency and committed cell lineages, with its levels decreasing during cell differentiation. Lin28 is undetectable in most differentiated cells but is often upregulated/reactivated in tumors where it acts as an oncogenic factor promoting cell proliferation and tumor progression.

In this study we investigated the structural and functional interactions between miR-125a and Lin28b in hepatocellular carcinoma cells. Firstly, we demonstrated the downregulation of Lin-28b by miR-125a and the effects of both the miRNA and Lin-28b on cell proliferation. Then HuH-7 cells were transfected with a Lin-28b expressing plasmid and we revealed the suppression of miR-125a by RT-qPCR. Based on the crystal structure of pre-let-7 bound to Lin28, we hypothesized that its Zinc Knuckle Domain (ZKD) may recognize a conserved GGAG motif of pre-miR-125a. HuH-7 cells were then transfected with plasmids driving transcription of pre-miR-125a with mutated GGAC or GGAA sequences. These mutations increased the amount of mature miR-125a thus confirming their weakening effect on the binding of Lin-28.

Overall, these data show a cellular pathway in which the reciprocal inhibition of miR-125a and Lin28b reasonably generates a positive feedback loop where decreasing levels of Lin28b further increase miR-125a expression thus potentiating its antiproliferative effect.

Adiponectin and Immunity: This Adiponekine may be a specific biomarker for CVID disease?

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Common variable immunodeficiency (CVID) is the most common primary immunodeficiency. Recently, we demonstrated that adiponectin and its HMW oligomers have a pivotal role in CVID. Adiponectin is strong associated to immune response, indeed it acts both innate and acquired immunity through the presence of its receptors, AdipoR1, AdipoR2 and T cadherin on B-cells, B-activated cells, NK cells but only small percentage of T cells. These findings led us to investigate whether adiponectin may be candidate for the CVID disease. We characterized by flow cytometry the peripheral blood leucocytes cells profiling as well as the expression of AdipoRs in CVID naïve patients before and after the first Ig- replacement therapy and by ELISA test, leptin and cytokines levels in CVID naïve patients before and after the first Ig-replacement therapy and in CVID patients in maintained therapy compared to healthy controls. **Our** analysis demonstrated that the expression (in terms of the percentage of positive cells) of AdipoRs is higher on B cells, B-activated cells and NK cells from treatment-naïve CVID patients before the first Ig-replacement therapy, and it is reduced after Ig-infusion. No expression of AdipoRs was found in T lymphocytes. Furthermore, cytokine and leptin serum levels are reduced in CVID patients compared to controls, in particular cytokine levels only slightly modified by IgG infusion but leptin levels, unlike adiponetcin, unchanged in CVID naïve patients. This is the first study that demonstrated that the expression of AdipoRs is strong modulated in treatment-naïve CVID patients compared to healthy controls. The deregulation of AdipoRs is associated with Ig replacement therapy and adiponectin levels supporting the "compensation theory" and confirms adiponectin as an important and specific factor in CVID. Further studies are needed to clarify the molecular mechanisms underlying adiponectin "system" and to confirm it as a biomarker in CVID.

The Lysophosphatidic Acid Acyltransferase (LPAATs) Enzymes and their Role in Membrane Transport Alterations in Cancer

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During tumorigenesis, the cancer factors secreted in the extracellular space are key actors in promoting the microenvironment required for tumor growth, migration/invasion. Indeed, the differences between the secretomes of cancer *versus* non cancer-cells, contribute to the extracellular microenvironment modifications that play a key role in tumorigenesis.

We have identified AGPAT4, a member of the 1-Acyl-Glycerol-3-Phosphate AcylTransferase family enzymes as an important controller of secretion in prostate cancer cells. Moreover, AGPAT4 expression/activation increases in prostate cancer and this correlates with tumor aggressiveness. Conversely, its depletion impairs cells migration/invasion. Thus, the study of soluble factors secreted under the control of AGPAT4 can be used to define the key factors involved in prostate cancer cells migration/invasion.

We performed a proteomic approach to identify factors differentially secreted in prostate cancer *versus* non-cancer cells, by LC-MS/MS. Then, we have identified, among these cancer-specific secreted factors, those with reduced secretion under AGPAT4 depletion following statistical data analysis with *Perseus* software. Among these proteins, we have selected those known to be secreted using *SignalP/SecretomeP* software and with increased expression in prostate cancer patients (using *cBioPortal for cancer genomics* data base).

The role of these AGPAT4-controlled secreted factors in prostate tumor migration and invasion are currently under investigation.

Session 4:

Human Genetics

The interplay of NEMO, RIPK1 and RIPK3 signaling in the regulation of cell death.

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Incontinentia pigmenti (IP), also known as Bloch–Sulzberger syndrome (OMIM #308300), is a neuroectodermal x-linked dominant genetic disorder with four distinct stages of skin eruptions in heterozygous females. It is a male-lethal disease with males usually die in utero. Despite IP is a male lethal disease, some affected males have been reported. Two mechanisms are responsible for survival of IP males: Klinfenter syndrome with 47, XXY karyotype which represent 7 % of all male cases and somatic mosaicism. 80 % of IP cases are caused by a mutation in the inhibitor of the kappa B kinase gamma gene (IKBKG, previously known as NEMO) located in locus 28 of the short arm of X chromosome. The pathogenic mutation is a frequent deletion of exon 4-10 of the IKBKG gene. IKBKG is responsible for activation of NF-kB pathway, a multicomponent pathway involved in a myriad of inflammatory, immune, cell survival and proliferation, cellular stress response and apoptotic pathways. It protects against TNF-α- induced cell death.
Several forms of cell death have been discovered and well characterized during the last years. One of the best studied form of cell death is Apoptosis, which can be defined as an ordered and controlled way to program a cell to die. Recently, another form of ordered necrosis termed necroptosis have been described. This form of cell death serves central roles in development, cancer pathology, immunity and degenerative diseases. It is regulated by receptor interacting protein kinase-1 (RIPK1), RIPK3, and mixed lineage kinase domain-like (MLKL).
Using overexpression and immunoprecipitation techniques, we showed a direct interaction between RIPK3 and NEMO and that this interaction is abolished in case of NEMO mutation A323P. WE also noticed that 2 population of cells are present: a population in which RIPK1 interacts with RIPK3 and the second one in which NEMO interacts with RIPK3. What functions are served by this interaction is still yet to be discovered and whether RIPK1 is interfering with NEMO-RIPK3 interaction is yet to be tested.
Understanding the functions of these interactions would provide a better understanding and dissemination of the molecular interplay between NF-kB and cell death mechanisms in determining cell fate in response to ligands like TNF. Especially, whether necroptosis might contribute to the pathogenesis of IP.

RIPK3 activity modified by NEMO and in IP cells? RIPK3 inhibitors effective in IP cells?

Characterization of murine models in imprinting disorders

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Heritable patterns of gene expression that does not involve changes in DNA sequence are the focus of epigenetics. Genomic imprinting is an example of epigenetics where some genes are expressed in a monoallelic and parental-origin-specific manner. Imprinted genes are usually involved in growth, behavioural and developmental control.

A 1 Mbp cluster of imprinted genes is localised at the human chromosome region 11p15.5 and organised in two domains, each containing an own imprinting control region (ICR or IC), *cis*-regulatory elements that show allele-specific DNA methylation.

Altered methylation of *H19/insulin-like growth factor 2* (*Igf2*) imprinted locus (IC1) is the one of the most frequent defects associated with two fetal growth disorders: Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS). The two syndromes are characterized by opposite growth phenotypes, growth retardation and overgrowth, respectively.

A mouse line has been previously generated as a model of both disorders. These murine knock-in models are characterized by the replacement of the endogenous mouse IC1 (mIC1) with orthologous human IC1 (hIC1) allele. This allele carries a mutation (hIC1Δ2.2), previously found in familial BWS cases. Interestingly, this knock-in mouse line shows pre/post-natal overgrowth on maternal transmission (BWS-like) and pre/post-natal undergrowth on paternal transmission (SRS-like).

The aim of the project is to analyze the phenotype of knock-in and control mice by histological analysis of different organs, including liver, kidney, tongue, brain, heart, lung and, possibly, to investigate the physio pathological mechanisms responsible of this phenotype.

Our preliminary results indicate that in knock-in mice kidney and liver display a lower number of hematopoietic nodules compared to controls. This result lays the groundwork for investigating the molecular mechanisms underlying these differences.

Histone modification controls subcutaneous adipose tissue hypertrophy on the way towards type 2 diabetes

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Background and aims: Subcutaneous adipose tissue (SAT) hypertrophy is associated with insulin resistance and increased risk of type 2 diabetes (T2D) and predicts future development of T2D independent of obesity. In humans, SAT hypertrophy is a consequence of impaired adipocyte precursor cell recruitment into the adipogenic pathway. This restricted adipogenesis might be due to epigenetic modifications. We aimed to identify the histone modification profiles in T2D First Degree Relatives (FDR), which are commonly characterized by inappropriate hypertrophy of the SAT, using a wide-genome approach.

Materials and methods: Genome-wide chromatin-immunoprecipitation sequencing approach (ChIP-Seq) for Tri-methylation of lysine 4 on histone H3 was applied in SAT Stromal Vascular Fraction cells obtained from 7 lean FDRs and 9 lean control subjects. Bioinformatic analyses were performed to determine differentially enriched regions (DERs) between the two groups. DERs were evaluated for potential overlaps with biological signaling pathways.

Results: Based on the bioinformatic analyses, we found 2644 DERs in FDR and control individuals. Intriguingly, we observed the altered regulation in genes belonging to the adipocyte differentiation and mitochondrial function pathways. These alterations, regulated by histone modification, could underlie SAT hypertrophy. The validation of differentially regulated genes is ongoing.

CRISPR-Cas9 neuronal cell model to investigate autophagy in neurodegeneration

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Progranulin (*GRN*) gene is one of the major genetic determinants of Frontotemporal Dementia (FTD) and mutations are present in 23% of familial cases. The neurobiology of the PGRN protein remains still unclear although the proposed disease mechanism is neuronal deficiency. We performed a genetic screening on 256 FTD patients and 300 healthy, age-, sex- and geographic region-matched controls and identified a rare *GRN* gene exon six deletion causing deficiency of the protein. To gain a system level view of the molecular consequences of PGRN depletion we used CRISPR-Cas9 to generate a cellular model of PGRN deficiency. We have evidences that in neuronal cellular models edited by CRISPR-Cas9, progranulin gene (*GRN)* is involved in macroautophagy. We analyzed the effect of PGRN protein depletion in mouse embryonic stem cells on mTOR dependent regulation of autophagy and identified a dysregulated molecular network. We are currently verifying the effect of PGRN depletion on stem cells differentiation in neurons to evaluate if autophagy dysregulation can affect cell fate and neuronal morphology. Our data show that autophagy dysfunction may represent the specific pathological mechanism underlying PGRN related neurodegenerative disorders.

Investigation of ICR2 epimutation in Beckwith-Wiedemann Syndrome

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The Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome caused by dysregulation of the imprinted gene cluster at human chromosome 11p15.5. The cluster is organised in two imprinted domains, where the monoallelic and parent-of-origin dependent expression of the imprinted genes is controlled by two different Imprinting Control Regions (ICR1, ICR2). The ICRs are genomic regions of ~ 2-4 kb in length, characterized by differential DNA methylation between the two alleles. Abnormal DNA methylation at the ICRs represents the most frequent molecular defect found in BWS, in particular loss of maternal methylation of the ICR2 (ICR2 LOM) is found in about 50% of the cases. One-third of them also show methylation abnormalities at other ICRs. So far, only rare genetic variants associated with ICR2 LOM have been described while in most of the cases the aetiological mechanisms underlying this epimutation are still undefined.

Aim of the project is to unravel the nature of the epimutation in BWS cases with ICR2 LOM, in order to distinguish the primary epimutations, not associated to DNA sequence variants, from the secondary epimutations, resulting from genetic mutations affecting *cis*-acting elements or *trans*-acting factor involved in the establishment or maintenance of imprinted methylation.

To reach our aim, the genotype (11p15 haplotype by microsatellite analysis), epigenotype (ICRs methylation by pyrosequencing) and phenotype (presence of clinical signs) will be analysed and compared between the patients and their siblings. Since the genetic mutations acting in cis or in trans are associated with higher recurrence risk than the primery epimutations, our study will be very important for genetic counselling.

Neuroimmune overlapping mutations leading to dementia: focusing on CD33 and TREM2 genes

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Alzheimer's disease (AD) and Frontotemporal dementia (FTD) are underlying pathologies with many candidate genes, including the immune genes *CD33* and *TREM2*, suggesting that a common molecular pathway could exist and is yet unknown. Two *CD33* SNPs (rs3865444 and rs12459419) and one in *TREM2* (rs75932628) were described to be predisposing factors to Late Onset Alzheimer disease (LOAD). We molecularly characterized affected patients from familial and sporadic cases for the presence of SNPs by High Resolution Melting Analysis (HRM) and we identified individuals carrying single and combined mutations in these genes, offering us a unique opportunity to identify their roles as disease modifiers. Our patients exhibited the coinheritance of SNPs rs3865444 and rs12459419 and not association with the risk allele of *TREM2* SNP rs75932628. In addition, we identified a third SNP in *CD33* exon 2, rs2455069, which belongs to a SNPs block associated with cognitive decline which we found in an unusual family with phenotype AD/FTD. Finally, we will create a patient-derived disease model generating Induced Pluripotent Stem Cells (iPSCs) from peripheral blood mononuclear cells (PBMCs) of patients carrying mutations in both genes and differentiate them into neurons and microglia in order to elucidate the common molecular pathways underlying AD and FTD.

Dissecting the molecular mechanism underlying Paget's Disease of Bone complicated by Osteosarcoma

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Paget's disease of bone (PDB) is a focal disorder of bone metabolism, involving both increased bone resorption and formation. Nearly 1% of pagetic patients develop secondary osteosarcoma (OS) in the affected bone areas, making the disease life-threatening and with a poor prognosis. Although the genetic alteration of PDB has been partially highlighted with the identification of *SQSTM1* mutations, the genetic defect underlying the pathogenesis of pagetic osteosarcoma (OS/PDB) has never been elucidated.

In this study, the exome sequencing analysis, performed on a pedigree with a familial clustering for OS/PDB, allowed us to identify a heterozygous loss of function mutation in the *PFN1* gene as the genetic cause for PDB, while the additional loss of the wild type allele was the basis for the OS transformation in a PDB member. We also disclosed the loss of the *PFN1* gene copies in 60% of sporadic OS/PDB patients, further correlating the osteosarcoma degeneration to its suppression.

To functionally prove the role of *PFN1* in OS/PDB onset, we used the CRISPR-Cas9 technology to generate mouse *Pfn1* knock-out cell lines. We demonstrated that the depletion of *Pfn1* in mesenchymal cells resulted in reduced levels of F-actin and focal adhesions, and consequent enhanced migration capability. Moreover, cells fail to complete abscission during late cytokinesis, containing up to 4 nuclei, and ploidy changes are a frequent hallmark of malignant cells. Furthermore, monocytes lacking the profilin-1 readily differentiated into mature osteoclasts and showed a greater resorption capability, explaining the enhanced bone destruction that typically characterizes PDB.

Towards the identification of new therapeutical compounds for a malignant epileptic encephalopathy caused by mutations in *Aristaless-related homeobox* gene

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Epileptic encephalopathies (EEs) are chronic neurodevelopmental disorders (NDDs) characterized by recurrent spontaneous seizures generally resistant to combinations of traditional anti-epileptic drugs (AED), severe neurological deficits, and sometimes early death.

Expansions of polyAlanine repeat tracts in the Aristaless-related homeobox (*ARX*) gene are responsible of a catastrophic EE syndrome with onset in the neonatal period of male patients. These mutations are produced by expanded runs of consecutive mixed (GCN)n repeats and cause a partial loss in ARX function, whose protein acts as homeotic brain transcription factor with a key role in mammalian corticogenesis and neuron maturation. Expanded ARX proteins produce a defective functioning of the epigenetic axis KDM5C-H3K4me3, whose alterations are emerging as a common feature in multiple NDDs.

We propose here the identification of new compounds, which activity could ameliorate and/or correct molecular and functional defects found in the polyAlanine repeat mouse model, $Arx^{(GCG)7/Y}$, which expresses seven GCG-triplets inserted at residue 330 of the mouse *Arx* gene. This model showed severe spontaneous seizures, a phenotype that well recapitulates the chronic epilepsy associated to c.304ins(GCG)7 in human patients. Specifically, we will test natural small molecules, already selected in our laboratory, in $Arx^{(GCG)7/Y}$ primary neurons and young animals, establishing their impact on ARX-related pathways. Ongoing efforts to identify new disease biomarkers may help to define drug-response and accelerate the discovery of innovative therapeutical compounds for malignant EE.

de novo mutations? haploinsufficiency? relationship with KDM5C?

Reactivation of the dormant wild-type allele of MECP2 as a therapy for Rett syndrome: screening of epigenetic compounds

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Heterozygous mutations in the MECP2 gene cause Rett syndrome (RTT), a severe neurodevelopmental disorder. Because MECP2 is X-linked, its allele-specific expression pattern depends on X-chromosome inactivation process. As a result, most female patients with RTT are somatic mosaic with approximately 50% of cells carrying the wild type but silenced allele of MECP2 on the inactive X chromosome. To monitor the allele-specific expression of Mecp2, we are generating mice carrying a double autofluorescent reporter system, where different tags are inserted within each allele of Mecp2 (XMecp2:eGFP/XMecp2:mCherry). We use mouse embryonic fibroblasts to establish a reporter cell system isolated from XMecp2:eGFP/XMecp2 female embryos. However, due to the low expression of Mecp2 in non-neuronal cells, the Mecp2:eGFP transgene-driven weak autofluorescence made arduous the physical separation by FACS of the Mecp2:eGFP+ MEFs from the Mecp2:eGFP- MEFs and the two subpopulations were almost impossible to be detected and distinguished at the microplate reader integrated to the Cellmaker after sorting, thus making reactivation events in Mecp2:eGFP- MEFs impossible to capture. To improve this issue, we decided to shift toward neural cells differentiated from mouse embryonic stem cells as an alternative.

examples of X-gene-specific activation, DNA methylation, chromatin modification, RNA?

Session 5:

Cancer biology, Immunology, Microbiology, Drug design

“Antarctized” antibody: an innovative engineered antibody by the CRISPR/Cas9 system

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Immunoglobulin (Ig) of Antarctic fish possesses some unique features in crucial parts of the molecule, such as a long hinge region, between the second and third heavy chain constant domain, and up to four short repeats at the extracellular membrane proximal domain. These peculiar structural characteristics, not found in any other vertebrate Ig, can be considered a result of adaptive evolution to improve the functionality of the molecule under very extreme environmental conditions.

These findings prompted me the idea to modify mouse monoclonal antibody by inserting the Antarctic Ig structural features by using the CRISPR-Cas9 system and test them for their impact on the structure and function of the Ig molecule. I will adopt the CRISPR/Cas9 technology since it allows the genome editing for the production of recombinant antibodies in a more efficient and accurate way than the commonly used systems.

Once obtained, the engineered monoclonal antibody will be characterized for either its structure (flexibility) or effector functions (antibody-dependent cell-mediated cytotoxicity; complement-dependent cytotoxicity) in comparison to wild type counterpart. Overall the results of my PhD project could be a promising starting point for future therapeutic or other biotechnological applications.

Modulating innate memory to treat inflammatory diseases

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Recent evidence showed that innate immunity possesses some degree of specificity in pathogen recognition, and is capable of displaying partially-specific memory. Nowadays innate immune memory is recognized as a crucial event in mammalian host defense, and many researchers worldwide are trying to better understand the molecular mechanisms that underlie it. Glycerophosphoinositol (GroPIns), a ubiquitous bioactive compound of eukaryotic cells, displays the potential to play a central role in innate and inflammatory reactions. Studies on macrophage-like cell lines reported increased amount of GroPIns when the cells are exposed to inflammatory stimuli (1–3). More recent data point to an anti-inflammatory role of GroPIns, at least as paracrine factor, since, in human primary monocytes stimulated with LPS and treated with GroPIns, GroPIns triggers a pathway that culminates in a decrease in inflammatory gene activation (4). Establishing the role of GroPIns in innate memory will support the possibility of its pharmacological exploitation as an anti-inflammatory drug and memory-modulating treatment in the therapy of chronic inflammatory and autoimmune conditions. My goal is to disclose the role of GroPIns in human primary monocytes and monocyte-derived macrophages, in a number of *in vitro* models of inflammation and polarization (5) and innate memory (6).

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Optimization of adoptive T cell therapy by promoting the correct pairing of T cell receptor chains

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The infusion of T lymphocytes directed against tumor antigens, or adoptive T cell therapy, has shown effective results in the treatment of melanoma patients. This approach is characterized by the engineering of autologous T cells introducing a tumor-specific T cell receptor (TCR). Despite the clinical efficacy, this therapy presents some drawbacks resulting in low expression of therapeutic TCRs and in a potential autoimmunity due to hybrid receptors that may form after the mispairing between the introduced and the endogenous TCR chains. To avoid this phenomenon, we propose to introduce aminoacidic mutations in the transmembrane regions of the transduced TCR chains in order to favour their correct pairing. Previously we observed a preferential pairing between mutated TCR chains that results in higher functional activity of murine T cells. Then, translating this strategy to human TCRs additional mutations were introduced in the membrane proximal domain of both chains, and during this year the study was focused on two TCRs specific for the melanoma antigen MART-1. We demonstrated that the proposed strategy is really effective since the measured TCR expression and the cytotoxic activity of transduced T lymphocytes are sensibly increased, indicating that our approach is a feasible improvement of the current therapeutic approach.

Characterization of an efflux pump in *Mycobacterium smegmatis*

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Tuberculosis (TB) remains one of the most important infectious disease worldwide, killing more than 1.5 million people each year. It is still endemic in many low and middle-income countries and the emergence of Mycobacterium tuberculosis multi-drug resistant strains continues to plague TB control. M. tuberculosis has the remarkable capacity to survive within the hostile environment of the macrophage; the molecular mechanisms behind the success of this pathogen are still poorly understood. Resistance to antimicrobial molecules represents a common strategy among different strains of M. tuberculosis for survival within macrophages. We recently described the role of a TetR-like protein of M. smegmatis and M. tuberculosis in regulation of the MSMEG_3762/63/65 and Rv1687c/86c/85c operons, respectively, coding for efflux pumps, using a combination of mutagenesis, local and global gene expression analyses and DNA binding studies. In M. smegmatis, MSMEG_3762 and MSMEG_3763 are annotated as ABC transporter ATP-binding protein and ABC transporter, respectively, as well as their orthologues in M. tuberculosis. In this contest, we have isolated a strain carrying a deletion in the MSMEG_3763 gene, and phenotypes related to efflux-mediated resistance to several anti-TB drugs and to acid-nitrosative stress, mimicking the macrophage environment, are under investigations.

New approaches to immunotherapy of inflammation through the use of engineered nanoparticles (ENP)

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Innate immunity is the major surveillance system tasked with maintaining the integrity and functionality of living organisms. Cells of the innate immune system, such as macrophages and monocytes, bear the responsibility for recognition and elimination of foreign threats against which the body must defend itself. We have endeavoured to exploit engineered nanoparticles in concert with these cells, as a venue for study of the mechanisms underlying phagocytosis, gene expression, and production of inflammatory proteins such as cytokines. We have developed models of *in vitro* culture based upon the kinetics of a physiological inflammatory reaction, in which monocytes or macrophages are exposed to nanoparticles and then subjected to a simulation of an immune response, which is based upon temperature, CO_2, and protein signals. Through use of nanoparticles in these systems, we are able to make observations based upon particle composition, shape, size, and surface functionalization. Preliminary data indicate that phagocytosis of a nanoparticle does not necessarily lead to a measurable immune response, and that composition or surface characterization are the important factors to consider. We further find that depending on experimental conditions, particles that may not otherwise induce an immune response, may initiate innate immune memory, altering the reactive potential of macrophages.

COMET: a novel oncogenic long non-coding RNA that regulates MET in papillary thyroid carcinoma

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BRAF and *RAS* mutations drive differential pathway activation in papillary thyroid carcinoma (PTC) and define two main tumor subgroups, i.e. *BRAF*- and *RAS*-like, respectively. Although the transcriptional signature for protein-coding genes in PTC has been characterized (Cancer Genome Atlas, 2014), the expression of long non-coding RNAs (lncRNAs) has not been systematically verified.

During my PhD, I analysed lncRNAs' expression in PTC to characterize those with oncogene-like or tumor-suppressing activity. In particular, I focused on a novel lncRNA, *COMET,* transcribed on the opposite strand of *MET* oncogene.

COMET and the neighbour *MET* oncogene are significantly over-expressed in *BRAF*-like samples compared to *RAS*-like and healthy controls. Perturbation of Mitogen-Activated protein kinase (MAPK) pathway revealed *COMET* as a downstream effector of MAPK signaling. Additionally, following *COMET* knock-down in tumour cells, a marked reduction of MAPK-related oncogenes, and in particular of *MET*, was observed. Accordingly, *COMET* repression increased apoptosis and inhibited viability and proliferation of tumour cells, dramatically reducing also colony forming ability, motility, invasiveness and the epithelial-to-mesenchymal transition of tumor cells. More interestingly, silencing of this lncRNA markedly increased sensitivity to vemurafenib, a common drug used as inhibitor of mutated B-raf. Taken together, these results suggest *COMET* as a lncRNA with oncogenic properties in thyroid and as a new potential target to improve drug-based therapies, especially in *BRAF*-mutated and *MET*-addicted tumours.

RNA fractionation and RNA FISH highlighted its cytosolic enrichment, suggesting its *trans* mode of action. Further studies, including RNA pull-down followed by mass spectrometry, to mechanistically define *COMET* function are in progress.

Analysis of expression of HLA class II risk alleles in Celiac Disease

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The Human Leukocyte Antigen (HLA) represents the main genetic risk factor for autoimmune disorders.
In celiac disease (CD), an autoimmune pathology that is triggered by the ingestion of gluten, the 95% of patients carry the HLA-DQA1*05 and DQB1*02 predisposing alleles, encoding the DQ2.5 molecule.
The DQA1*05 and DQB1*02 alleles are located on chromosome 6 either in *cis* (DR3/DRX) or *trans* (DR5/DR7) configurations. These CD risk alleles, in both haplotypes, are more expressed than the non-CD predisposing alleles, favoring the gliadin antigen presentation and the establishment of autoimmune response.
In order to investigate the molecular mechanism that controls the differential expression of risk alleles, we have analysed the transcriptional regulation through click chemistry. This method consists in the use of 5-ethynyl Uridine (EU), an analog of uridine, which is incorporated into the nascent RNA. By a biotin-based handle, *de novo* transcripts are captured on streptavidin magnetic beads and used as template for cDNA synthesis. The amount of each mRNA is measured by qRT-PCR using allele-specific primers. Preliminary results suggest that the high expression of CD-associated DQA1*05 and DQB1*02 risk alleles, respect to non-CD associated ones, is mainly determined by a difference of transcription rate.

Relationship between resistant hypertension and coronary vascular function with ^{82}Rb PET/CT in patients with suspected CAD

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Background: Impaired coronary flow reserve (CFR) is a biomarker of hypertensive vascular damage and indicate a condition of increased risk of cardiovascular morbidity and mortality. In patients with resistant hypertension (RH) reduction of CFR may occurs earlier than coronary atherosclerosis. Therefore, we assessed the relationship between RH and CFR assessed by ^{82}Rb PET/CT.
Methods: The overall study population consisted of 87 subjects with hypertension and normal myocardial perfusion imaging assessed by stress-rest ^{82}Rb PET/CT. RH was defined as blood pressure ≥140/90 mmHg despite the concurrent use of 3 antihypertensive agents. Absolute myocardial blood flow (MBF) was computed from the dynamic rest and stress imaging series. CFR is the ratio of hyperemic to baseline MBF and CFR ≤2 was considered reduced.
Results: In the overall population, 36 patients (38%) had RH, they were significantly older and showed significantly lower global hyperemic MBF and CFR as compared to those without RH (all $p < .005$), while no differences were observed on the baseline MBF.
At univariable linear regression analysis age and RH resulted significant predictors of decreasing CFR (all $P < .01$). However, at multivariable analysis, only RH was independently associated with decreasing CFR ($P < .05$).
Conclusions: RH resulted independent predictor of impaired CFR.

Role of PAX8 in the Fallopian tube epithelium, the site of origin of HGSC

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High Grade Serous Ovarian Carcinoma (HGSC) is one of the most aggressive and lethal gynecological malignancies attributed by its late diagnosis and absence of early stage markers. Recently, the origin of HGSC has been attributed to transformation occurring in the fallopian tube epithelium (FTE) supported by PAX8 expression in both. PAX8, a member of the Paired – box gene family of transcription factors, is an important histological marker of HGSC and also the lineage-specific marker of FTE secretory cells. Therefore, it is crucial to explore PAX8's role in the development of FTE for understanding its progression to HGSC. This would be done by analyzing the physiology of FTE using immunofluorescence (IF) in PAX8 knockout transgenic mice. The structural, morphological, histological differences between normal ($PAX8^{+/+}$) and heterozygous ($PAX8^{+/-}$) knockout mice has been analyzed using H&E staining and Immunofluorescence (IF). The physiology of FTE in null ($PAX8^{-/-}$) mice should be further examined. To understand the progression from FTE to HGSC, RNA-sequencing analysis of FT-194 (FTE secretory cell line) and SKOV-3 (Ovarian cancer cell line) before and after PAX8 silencing, was previously performed in our lab. Amongst the differentially altered transcriptional expression between FT194 and SKOV-3, several lncRNAs were identified. Currently, we intend to identify certain candidate lncRNAs using bioinformatic analysis and validate them in a panel of HGSC cell lines, FT cell lines, primary FTE cells and organoid models.

PDGFRβ as a new biomarker for metastatic triple-negative breast cancer: development of a theranostic anti-PDGFRβ aptamer for imaging and suppression of metastases

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Triple-negative breast cancers (TNBCs) are a heterogeneous group of aggressive tumors lacking estrogen and progesterone receptors and HER2 receptor, thus excluding the possibility of using targeted therapy against these proteins. Mesenchymal-like (ML) subtype, characterised by a stem-like, undifferentiated phenotype, is more invasive and metastatic than other TNBC subtypes and has a strong tendency to form vasculogenic mimicry (VM). Recently, platelet derived growth factor receptor β (PDGFRβ) has been shown to play a role in VM of TNBC. Regrettably, therapies targeting PDGFRβ with tyrosine kinase inhibitors are not effective in treating TNBCs, thus developing new strategies to target PDGFRβ in TNBC patients is crucial to improve their chances of survival. Here, we describe the characterization of the Gint4.T nuclease-resistant anti-PDGFRβ RNA aptamer as high efficacious theranostic tool for imaging and suppression of ML TNBC metastases. The expression of PDGFRβ was observed in few cases of human TNBC samples, characterized by higher metastatic behavior. Treatment of TNBC cell lines with Gint4.T aptamer blocked their invasive growth and vasculogenic properties in 3D culture conditions, and strongly reduced cell migration/invasion *in vitro* and metastases formation *in vivo*. The Gint4.T-NIR was able to specifically bind to TNBC xenografts and detect lung metastases *in vivo*. Therefore, the aptamer revealed a high efficacious theranostic tool for imaging and suppression of TNBC metastases. These studies indicate PDGFRβ as a new biomarker for ML and metastatic TNBC subtype and propose a novel-targeting agent for the diagnosis and treatment of metastatic TNBCs.

Development of MRI-based pH imaging as biomarker of treatment response

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Solid tumors are characterized by the constitutive upregulation of glycolysis that leads to excessive production of acidic metabolic products that accumulate in the cytoplasm. Protons generated from these products lead to an acidosis condition in the extracellular environment due to the presence of several H+ transporters. Clinical investigations have shown that tumors with acidic environment are associated with poorer prognosis and an enhanced metastatic incidence, increased mutation rate and resistance to chemotherapy and radiotherapy. Because survival in the tumor microenvironment depends on the control of the homeostasis of pH, interference with pH regulating systems is now considered a relevant therapeutic goal. In this project three human prostate cancer cell lines with different metastatic potential (PC3, LnCap and DU145) will be used and then treated with several proton pump inhibitor to assess the capability of these drugs to prevent the acidification of the extracellular pH (pHe). In particular, five drugs (lansoprazole, esomeprazole, acetazolamide, cariporide and amiloride) that target three proton transporters, namely CAIX, NHE-1 and V-ATPase were chosen. *In cellulo* experiments were done varying several conditions (normoxia and hypoxia, different drug concentrations, two time points) to establish both survival rate with MTT assays and pHe variations with fluorescence assays. The obtained results showed an heterogenous response to drug treatment for both the cell toxicity and for pHe variations. Following these studies, the more efficient drugs for affecting pHe will be selected for further in vivo experiments. Both xenograft and orthotopic tumor models will be investigated and the in vivo efficacy of these drugs will be monitored by using an MRI-CEST (chemical exchange saturation transfer) pH imaging approach.

Exploitation of new strains for drug discovery from deep sea sediments

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Introduction Currently, multi drug resistant infections are a primary concern for the WHO and society as a whole. Microorganisms, such as human pathogenic bacteria have developed resistance to antibiotics primarily through the misuse and overuse of these drugs. Extreme environments such as the Antarctic, harbour a diverse range of microorganisms that have over the course of evolution developed molecular adaptations to these cold environments. The unique metabolism of these microorganisms includes secondary metabolites that have been reported to possess several bioactivities, including anti-microbial and anti-biofilm activities. In this PhD study, microbial diversity and metabolic potential of Antarctic deep sea sediments, are assessed for pharmaceutical and biotechnological applications. **Methods** Deep-sea sediments were sampled from 2000-5000 m from a previously unexplored environment: the South Shetland trough, Antarctica. Bacteria were isolated from sediments using the following culture-dependent techniques: longer incubation time, lower temperatures, and different culture media. Isolates were identified by 16S rDNA sequencing and 6 were considered interesting based on taxonomy. These isolates were subjected to small-scale fermentations, and crude extractions using organics solvents. Extracts were assessed for anti-microbial activity using a liquid inhibition assay. **Results** 50 bacteria have been isolated using culture-dependent techniques. 6 isolates of interest were assessed for anti-microbial activity were subjected to fermentation, organic solvent extraction and bioactivity screening, but no significant activity was found. **Conclusion** 2-3 novel isolates will be subjected to *de novo* whole genome sequencing. The genomes will then be mined for biosynthetic gene clusters or pathways. Using the bioinformatics genome data, compounds of interest will be expressed or activated *in vitro,* followed by chemical characterization (e.g. HPLC/MS/NMR) and toxicity evaluation (e.g. zebrafish system).

Cannabinoids and bone: from metabolic to malignant diseases

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Bone is a metabolically active tissue, which is continually remodeled. The osteoclasts resorb bone whereas osteoblasts form new one. Imbalance between resorption and formation accounts for several diseases including Paget's disease of bone (PDB) in which tissue can undergo neoplastic transformation leading to giant cell tumor of bone (GCT/PDB) and osteosarcoma (OS/PDB). Recently, the germline mutation (P937R) in the ZNF687 gene as responsible for GCT/PDB was identified.
Cannabinoids, a heterogeneous group of compounds acting via specific molecular targets, have been shown to play a key role in the regulation of skeletal remodeling and bone mass as well as of cancer growth and spreading.
Project aims at investigating, via transcriptomic and targeted lipidomics analyses, the role of the endogenous cannabinoid system (ECS) in the Zfp687P937R knock-in mouse model of GCT/PDB to explore therapeutic potential of cannabinoids. We also investigate ECS role in osteoblast differentiation process, by using the wild type osteoblast precursor MC3T3-E1 cell line as well as the Zfp687 knock-in and knock-out MC3T3-E1 cells.
As metastatic prostate cancer cells and osteoblasts dynamically regulate each other, osteoblast proliferation and differentiation will be assessed via direct and indirect co-culture system with cells originated from a multi-stage model of prostate cancer.

PD1 increases stemness and proliferation in Thyroid Cancer Stem Cell through Ras activation

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Immunomodulatory molecules play critical role in immune surveillance. Alteration in the expression of these immunomodulatory molecules can change the immune functions. We characterizing the immunomodulatory molecule status in anaplastic Thyroid Cancer (TC) cells, papillary TC cells and immortalized normal thyroid (Nthy) cells. Immunomodulatory molecules PDL1, PD1 and Ido were significantly over expressed in TC cells compared to Nthy cells. Immunomodulatory molecule expression was higher in anaplastic TC cells compared to papillary TC cells with correlated with the aggressive behavior of the cancer. To explore the effect of stemness on immunomodulation in TC cells, expression of these molecules at mRNA level was also analyzed which supported these results.
PD1 overexpressed TC cells showed increased growth and proliferation. PD1 overexpressed TC cells showed increased BrdU incorporation. We investigated for the signalling pathway involved in TC cell proliferation and found increased phosphorylation of MAP Kinase/ MEK/ AKT / S6 in PD1 transfected TC cells. This phosphorylation was increased in presence of EGFR co-transfection with PD1. We also found increased BRAF phosphorylation in PD1 transfected 8505c anaplastic TC cells. Upon investigation upstream signalling we found Ras activation in PD1 transfected cells. We investigated to dissect the molecular mechanism by which immunomodulatory molecules enhance the stemness in cancer cells and contribute to their proliferation. PD1 on the TC cell membrane binds to C-terminal SH2 domain of SHPTP2 this binding was increased in the presence of serum and epidermal growth factor (EGF). Increased binding of SHPTP2 with GRB2 and Gab1 was observed in PD1 transfected TC cells and this was enhanced in presence of EGF. In conclusion we found that PD1 increases proliferation of TC cells through SHPTP2 by activating Ras and its downstream signalling.

Pharmacological chaperones to cure genetic diseases

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Fabry Disease (FD) is a rare genetic disease caused by mutations in the *GLA* gene that encodes for a lysosomal enzyme: the α-Galactosidase A. There exist more than 700 missense mutations of *GLA*, causing the disease, that have different effect on the activity of the enzyme and different symptoms in patients with the consequent difficulty to predict the clinical outcome and to find a suitable therapy. Our study is focused on a possible therapy that uses small molecules called pharmacological chaperones (PC). PCs are able to bind the mutated enzyme, stabilizing it and increasing its concentration and then the enzymatic activity within the cell. The PC newly approved for FD that we tested is called DGJ (1-deoxigalactonojirimycine). The present work has been carried out at the "Albrecht Kossel Institute for Neuroregeneration" of the University of Rostock (Germany). This laboratory has developed a database containing all the missense mutations found so far in Fabry patients. 91 *GLA* mutants were obtained *in vitro* by PCR mutagenesis. 35 mutants of them were transfected in HEK293H cells, treated with DGJ and their α-Galactosidase activity was tested through a standardized and reproducible *in vitro* enzymatic assay. The used assay represents the basis of a pre-clinical screening to find out which patient is amenable for this PC therapy.

A proteomic approach identified HSP90 as a central hub in CDDP-resistant ovarian cancer cells

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Epithelial ovarian cancer (EOC) remains one of the leading causes of cancer-related deaths among women worldwide. Standard treatments for newly diagnosed cancer consist of cytoreductive surgery and platinum-based chemotherapy. However, acquired resistance to platinum occurs frequently and predicts poor prognosis. Therefore, understanding the mechanisms underlying platinum resistance and finding ways to overcome them are active areas of study in ovarian cancer. On this regard, we have generated and characterized three cisplatin (CDDP)-resistant isogenic high grade EOC cell lines (TOV-112D Mi-res, OVSAHO Mi-res and MDAH Mi-res) from their parental counterpart. To further investigate the mechanism by which cells acquire CDDP-resistance, we took advantage of the 2-D DIGE followed by LC-MS/MS to compare the protein expression profile of the three CDDP-resistant models compared to parental cells. We identified several differentially expressed proteins and specifically 23, 24 and 20 for TOV-112D, OVSAHO and MDAH respectively. The IPA analysis showed a relevant relationship between almost all the identified proteins in the three models. Interestingly, eight of the identified proteins, shared at least within two cell lines, are related in one main network. Multiple central nodes, namely GRB2, p85, p110, AKT and HSP90 were identified in the protein-protein networks related with cancer. In particular, the protein chaperone HSP90 emerged as a central hub and its activation, evaluated in terms of increased phosphorylation levels, was observed in the CDDP-resistant cells.
Given that numerous oncoproteins have been identified as HSP90 clients, HSP90 inhibitors have the potential to block multiple oncogenic pathways as well as drug resistance in several cancer types. On this regard, we are going to evaluate the effect of CDDP in combination with two HSP90 inhibitors: 17-AAG and ganetespib, both of them in phase II/III in cancers patients.

Cannabinoids as metabolic reprograming agents in prostate cancer cells

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Cancer is characterized by uncontrolled proliferation of cells, invasion of neighboring tissues and metastasis. As an additional hallmark, all types of cancers are linked to impaired mitochondrial function and dysregulated energy metabolism generally supporting a view of cancer as a mitochondrial metabolic disease. Cannabinoids have been reported to affect mitochondrial functions.

We previously demonstrated that CBD (alone and in combination with CBG) significantly reduced tumor progression in TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice, which uniformly and spontaneously develop multistage autochthonous (orthotopic) prostate tumors following the onset of puberty. Cannabinoids were also able to block tumor relapse in an *in vivo* model of castration resistant prostate cancer (CRPC) settled in TRAMP mice exposed to Enzalutamide, a second-generation androgen receptor antagonist.

Here, we investigated whether cannabinoids act as metabolic reprogramming agents in enzalutamide-resistant phenotype of TRAMP-C2 cells (an *in vitro* model of CRPC), which showed unique metabolic features. Both cannabinoids were anti-proliferative and exerted a pro-apoptotic effect. NMR-based metabolomics and extracellular flux analysis indicate that CBD induces glycolysis and inhibits glutaminolysis in these cells.

Overall, we found that CBD and CBG were more efficacious in affecting respiration rates and mitochondrial functions on CRPC-TRAMP-C2 cells rather than on their parental counterpart.

Repurposing of valproic acid, simvastatin and aspirin combination as anticancer agents

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Drug repositioning, also named "drug repurposing" is a promising approach to overcome hurdles in discovering and developing new drugs via identification of the new therapeutic applications of known drugs.
Cancer is one of the most complex diseases as well as the leading cause of death worldwide. While better screening methods and treatments are saving lives, some tumor types remain terribly lethal. Among them pancreatic cancer is one of the deadliest tumors with 5-year survival rates still under 10%, similar low rates of survival were observed for metastatic prostate cancer or triple negative breast cancer. Few drugs have been able to make significant improvements in patient outcomes, particularly in advanced settings and, albeit a growing numbers of new agents are available for clinical studies, many fail in phase 2/3 clinical trials. In light of this situation, repurposing the vast arsenal of non-oncology already-approved drugs , that have existing preclinical and clinical safety data available, might be an attractive strategy to offer more-effective treatment options to patients with cancer.
In this scenario, we focused our attention on aspirin, statins and valproic acid. Emerging evidences suggest an anti-cancer mechanism of aspirin, based on the inhibition of COX-2; of statins, lipid-lowering agents that have demonstrated on both in vitro and in vivo studies an anti-proliferative, anti-metastatic, RT-sensitizing, and apoptosis inducing properties; and finally of the short chain fatty acid, valproic acid, an inhibitor of class I histone deacetylases with numerous mechanisms of anti-cancer effect.
Preliminary data show a synergistic effect of valproic acid and statins in pancreatic cancer, demonstrated as a reduction of cell growth and colony numbers after drug treatment.
Our goal will be to evaluate novel combination strategies using these approved drugs in association with chemotherapy to select new therapeutic options and potential biomarkers to select patients that will benefit of these approaches.

The Urokinase Receptor Antagonist RI-3 potently inhibits sarcoma cell invasion in a 3D organotypic co-culture model.

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The receptor of urokinase (uPAR) is a recognized master regulator of cell migration and $uPAR_{88-92}$ is the minimal sequence required to induce cell motility by interacting with the formyl peptide receptor type-1 (FPR1). The uPAR-derived retro-inverso Ac-(D)-Tyr-(D)-Arg-Aib-(D)-Arg-NH2 (RI-3) peptide adopts the turn structure typical of the uPAR-FPR1 antagonists, is stable in human serum and inhibits in vitro and in vivo migration and trans-endothelial migration of sarcoma cells. Tumor cells grow in a three-dimensional (3D) environment in which a mutual interaction exists between tumor cells and cellular and non-cellular components of the tumor microenvironment. To investigate the effect of RI-3 on the invasive capability of sarcoma cells, we have setup a 3D organotypic co-culture model which is highly comparable to the in vivo tumor tissue. By embedding preformed sarcoma spheroids into a collagen matrix contracted by fibroblasts, we found that spheroid size increases over time in matrices. Sarcoma cells and fibroblasts spread in the matrix, moving towards each other already after 24 h co-culture, continuing to spread up to 7 days and the addition of RI-3 caused an appreciable decrease of spheroid size and spreading of sarcoma cells into matrices. Thus, RI-3 represents a promising lead for developing novel anti-metastatic drugs.

Drug-discovery from marine natural compounds

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The aim of this research project is to identify novel bioactive compounds from marine organisms (protists, bacteria and invertebrates) by the development and use of an innovative platform of drug discovery.
A drug screening will be performed using about 50 different biologic samples for finding novel bioactive compounds and testing the efficiency of the innovative platform of drug discovery.
The essential element of the platform consists in the fractionation of the crude extracts. The molecules are separated in five fractions of decreasing polarity in such way that the large salt quantities from marine organisms cannot interfere with the rest of organic compounds. Moreover, the compounds in the other fractions become very concentrated and can be tested with higher sensitivity.
The crude extracts together with their fractions are then used for identifying biologic activities, by testing them against a panel of cancer cell lines and measured the cytotoxic activity with a 48h mtt assay. This point was reached with most of the biologic samples, but some test still needs to be done for finishing all.
Further tests will be done to detect other biologic activities such as antibiotic or antiprotozoal.
The following steps consists in a reiterative bioassay guided fractionation to purify the compounds with interesting activity, dereplication and molecular elucidation.

Glioblastoma: molecular compounds targeting tumor cells

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Glioblastoma (GBM) is an aggressive grade-IV glioma. Uncontrolled cellular proliferation, aggressive invasiveness and hypervascularization are the major GBM pathological characteristics. GBM vascularization involves Vasculogenic mimicry(VM), which defines tumour cells ability to form blood vessels independently of normal endothelial and mural cells, a mechanism whereby GBM could escape anti-angiogenic therapies.
I evaluated whether molecules, such as Histone Deacetylase inhibitors (HDACis), or Ruta Graveolens extract affect glioma cell lines and primary neural cells.
Among HDACis, I tested non-toxic concentrations of SAHA, TSA, MS275 and MC1568 in Tube formation assays. Tubules formation by tumor cells is the mechanism underlying VM. All HDACis studied inhibit tube-like structures formation by U87MG cells, whereas only SAHA and MC1568 significantly decrease the tube formation by C6 cells. Moreover, HDACis significantly decrease U87MG directional cell migration and MS275 is able to decrease, also, U87MG matrigel invasion.
Ruta extract has proven capable to inhibit cell proliferation, motility and tube formation in GBM cells and treatment with lower concentration affecting proliferation do not affect neurons viability and promotes primary microglia activation.
Our results suggest that HDACis can be promising compounds to control GBM hypervascularization and that Ruta extract may be a promising new compound in pre-clinical research on GBM.

Identification and characterization of a Golgi glycosyltransferase as a new potential oncogene

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Aberrant localisation, expression and function of different Golgi-complex glycosyltransferases are hallmarks of many cancer phenotypes.
In our laboratory we recently discovered that the Golgi localized oncogene GOLPH3, that is often amplified in solid tumors exerts its oncogenic activity through the regulation of Golgi glycosyltransferases.
Specifically, we found that GOLPH3 positively regulates glycosphingolipid (GSL) synthesis by controlling the localization and stability of B4GALT5, a Golgi glycosyltransferase. The change in sphingolipid metabolism induced by the manipulation of GOLPH3 levels has an impact on a signalling pathway responsible for cell proliferation, thus influencing cell growth.
Interestingly, we found that B4GALT5, which is necessary and sufficient for the oncogenic effect of GOLPH3, is often amplified in the same tumour types where also GOLPH3 is amplified. Thus, we ask whether B4GALT5 is an oncogene.
Gain-of-function experiments in immortalized mouse fibroblasts (NIH3T3) shows that B4GALT5 promotes cell growth and proliferation *in vitro.*
Mechanistically, B4GALT5 by altering the glycosphingolipid metabolism affects the mTOR signalling in a phosphatidylinositol (3,4,5)-trisphosphate dependent manner.
Thus, genetic, functional and biochemical data suggest that B4GALT5 is a possible new oncogene in human cancer. Importantly, overall these data suggest that the inhibition of sphingolipid metabolism represents a valuable therapeutic option for cancer patients bearing the overexpression/ amplification of GOLPH3 or B4GALT5.

Development of multifunctional RNA-based therapeutics to selectively target the stem-like glioblastoma cancer cells

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The aim of this work is to address the epigenetic therapeutic targeting. To address this issue we decided to focus on **SALL4**, a transcription factor essential for the maintenance of pluripotency and self-renewal capacity of Embryonic Stem Cells (ESC). SALL4 expression is found to be restored in various cancers, among which glioblastoma, where it has been linked with pathology grade and poor outcome. It has been demonstrated that SALL4 acts as a transcription repressor of tumor suppressor gene (PTEN) by interacting with a histone deacetylase (HDAC) complex (NURD complex) and that blocking SALL4-NuRD interaction hampers its repressive function reversing the aggressive phenotype.
We are addressing SALL4 targeting by two different approaches. First strategy developed involves the blocking of SALL4 expression by means of short interfering RNA (siRNA). The novelty of this approach is the use of aptamers developed against membrane receptors overexpressed in glioblastoma cells as carriers for the delivery of therapeutic siRNA against SALL4. Second strategy focus on the selection of new aptamers against SALL4 by applying protein-SELEX (Systematic Evolution of Ligands by EXponential enrichment) in order to study the effect of blocking its interaction with NURD.

Identification of novel therapeutic strategies to treat neurodegenerative disorders

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Neurological disorders constitute over the 6% of the global burden of disease which represent a major public health problem. Those disorders are mainly characterized by a progressive loss of structure or function of neurons; depending on the type of disease, the neuronal dysfunction could cause dementia, motor dysfunctions and behavioural issues.
A common feature is the activation of immune response, in particular of microglia that are specialized macrophages resident in the Central Nervous System (CNS). Apart from the removal of damaged neurons and infectious material, microglia are also important for maintaining the health of the CNS (1). Microglia can secrete a bewildering number of pro-inflammatory mediators which, in case of pathological dysfunctions such as Alzheimer or Parkinson disease, can significantly contribute to neuronal degeneration and brain damage (2).
Herein, the modulation of microglia activation through specific receptors could attenuate the neuro-inflammatory process or even block neuronal damage. Our studies are aimed to screen several compounds on cell lines expressing microglial receptors that play a crucial role in immune response of different neuropathological dysfunctions. The mechanism of action of lead compounds will be further envisaged *in vitro* using human microglial cell lines. Finally, the effectiveness in inhibiting signs of degeneration will be tested *in vivo* on animal models of neurodegenerative disorders.

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Extreme environments as a source of new potential drugs

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The extreme environments are among the most diverse habitats on the earth. They show very different and sometimes harsh conditions such as changes in temperature, pressure, salinity, presence of light and oxygenation. For these reasons, these environments are inhabited by a wide variety of organisms that have developed unique metabolic abilities to ensure their survival, which have resulted in the biosynthesis of an array of secondary metabolites with unique biological activities.

Despite its variability and biosynthetic potential, the marine environment remains poorly explored, so in the last few years research is being focused on the identification of new molecules with important bioactivities, from the sea environment. The natural products present several advantages as compared with non-natural compounds such as high chemical diversity, biochemical specificity, binding efficiency and propensity to interact with biological targets, which make them favourable lead structures [2]. Currently, several natural products have already been recognized as anti-tumor, anti-proliferative, photoprotective, antibiotic, anti-infective, antifungal, antiviral, antiparasitic, anthelmintic [3].

Some of the bacteria, I am currently working on, have been isolated from sediments coming from Faro's lagoon, Portugal, and others from a Tibetan glacier. Fifty bacteria have been isolated and they have been screened for some biological activity, and the best candidates were identified by 16S rDNA sequencing. This was followed by small scale cultivations, liquid-liquid extractions of exhausted culture broth, and the extract was dried and tested for antimicrobial and antioxidant activities in particular. Finally, the active molecules will be purified by HPLC and identified through mass spectrometry and NMR analysis.

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High-throughput Tissue based Molecular Classification of Human Gastric Cancer

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Gastric cancer (GC) is the third leading cause of cancer mortality worldwide. The overall survival of Gastric carcinoma patients remain poor, despite improved control over known risk factor and surveillance. GC has recently been classified into several types on the basis of molecular characterization, and the new taxonomy has shown to have clinical relevance. A Western and Japanese classification system of gastrointestinal epithelial neoplasia results shows large differences among pathologists in the diagnosis of gastric cancer lesions. The technology required molecular classification, it is complicated and expensive, currently preventing widespread use highlight the need for the new classification, driven toward, and identification of potential therapeutic target. The Aim of the study is to develop common worldwide terminology for gastrointestinal epithelial neoplasia and investigate the co-relation between Subtypes, clinic pathological feature with survival outcome of the Patients by the used of High-throughput Tissue based technology. And identify the new therapeutic target by the new molecular classification of Gastric cancer.