Università degli Studi della Campania *Luigi Vanvitelli*

PhD Days 2020
PhD Programme in Molecular Life Sciences

Cancer biology, Immunology, Microbiology, Drug design

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Antarctized" antibody: an innovative engineered antibody by the CRISPR/Cas9 system
Tutor: Dott.ssa Maria Rosaria Coscia

Ana Margarida Ferreira Campos
Modulating innate memory to treat inflammatory diseases
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Rita Lombardi
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Chetan Bharatkumar Dhakan
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Tutor: Professor Silvio Aime; Dr. Dario Longo

Pietro Irrera
Using in vivo MRI-CEST tumor pH imaging to detect early resistance to proton pump inhibitors in human prostate cancer murine models
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Mariateresa Allocca
Search for orphan drugs using bioinformatics tool and in vitro validation
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Magdalena Kostrzewa
Effect of non-psychotropic cannabinoids on androgen deprivation therapy in high-fat diet-induced prostate cancer: implications for gut microbiome
Tutor: Dr. Fernando Gianfrancesco/ Dr. Alessia Ligresti

Mario Campanile
Effect of Ruta graveolens water extract on ischemic damage and neurological deficits in a rat model of transient focal brain ischemia
Tutor: Prof. Giuseppe Pignataro, Prof. Luca Colucci D'Amato

Ali Mokhtar Mahmoud
Effect of non-psychotropic cannabinoids on tumour growth in hormone refractory prostate cancer
Tutor: Dott.ssa Alessia Ligresti

Gemma Conte
Hybrid lipid/polymer nanoparticles for pulmonary delivery of siRNA in cystic fibrosis lung inflammation
Tutor: Prof. Ivana D'Angelo

Janardhan Ausuri
Isolation and characterization of PAH's degradaing bacteria by Dietzia and Rhodococcus sp.
Tutor: Dr. Donatella De Pascale

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 aptamer-based therapeutics to selectively target the stem-like cancer cells
Tutor: Dott.ssa Paola Ungaro

Marisa Saponaro
Identification of lead compounds from Marine Natural Products as novel therapeutic strategies to treat neurodegenerative disorders
Tutor: Dr. Carmela Gallo

Carmine Buonocore
Novel antimicrobial biosurfactants from Antarctica
Tutor: Dr. Donatella de Pascale

Giovanni Andrea Vitale
Marine environment as a source of new multiactivity pigments and bioactive molecules
Tutor: Dr. Donatella de Pascale

Henu Kumar Verma
Identification and characterization of Gm4340 (Thoc4) gene expression in mRNA Export in Embryonic Stem Cells
Tutor: Prof. Geppino Falco

Veronica Sarnella
Targeting CA IX prevents hypoxia-induced cell plasticity in Triple Negative Breast Cancer
Tutor: Zannetti Antonella

Chetan Dhakan
Preclinical assessment of Proton Pump Inhibitors by multi slice MRI-CEST pH Imaging: A study in metastatic breast cancer
Tutor: Dott. Silvio Aime

Federica Farina
Study of HLA-DQA1 and DQB1 risk alleles conferring genetic susceptibility to Celiac Disease
Tutor: Dott.ssa Giovanna Del Pozzo

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

Mariavittoria Laezza
Regulation of HLA DQ2.5 genes in subjects with high/moderate risk to develop Celiac Disease
Tutor: Dott.ssa Giovanna Del Pozzo

Gene Regulation and Computational Biology

Ankit Verma
Investigation on regulatory components and differentially methylated regions associated with genomic imprinting and imprinting disorders
Tutor: Prof. Andrea Riccio

Valeria Policastro
New statistical methodologies for Omic Network Integration
Tutor: Dott.ssa Annamaria Carissimo

Ichcha Manipur
Network similarity measures for tumor metabolic networks
Tutor: Dott. Mario Rosario Guarracino

Francesco Cecere
Bioinformatic analysis of RNA-seq data to identify molecular pathways involved in two imprinting disorders: Beckwith-Wiedemann and Silver-Russell syndromes
Tutor: Prof. Andrea Riccio

Carlo Giaccari
The role of Padi6 in genomic imprinting. A new mutant mouse model
Tutor: Andrea Riccio

Karla Ruiz
Identification of the gene networks that modulate ABCA4 expression
Tutor: Sandro Banfi

Varsha Poondi Krishnan
Integrated analysis of epigenomic and trancriptomic signature in patient derived iPSCs carrying DNMT3B mutations and their isogenic lines with restored DNMT3B following CRISPR/Cas9
Tutor: Dr. Maria R Matarazzo

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

Structure and Functions of Biomolecules

Nicola Landi
Determination of primary structure, gene organization and antifungal activity of Ageritin, the first ribotoxin-like protein from edible mushrooms
Tutor: Prof. Antimo Di Maro

Mariangela Valletta
Secretome analysis reveals a role of pentraxin-3 in modulating the angiogenic activity of high mobility group box 1 in breast cancer
Tutor: Prof. Angela Chambery

Manoj Madheswaran
Structural Analysis of Metal ion binding Human Prion Proteins using NMR Methodologies
Tutor: Prof. Roberto Fattorusso

Marica Sassano
PTFE nanoplastics impact on Bifidobacterium growth and biofilm formation.
Tutor: Gaetano Malgieri

Maria Della Valle
Characterization of micro- and nano-plastic interactions with biological systems through NMR methodologies
Tutor: Prof. Roberto Fattorusso

Andrea Corvino
Structural characterization of multi-domain proteins involved in neuronal diseases
Tutor: Roberto Fattorusso

Mario Di Gennaro
Preparation of devices from natural substances for controlled release
Tutor: Prof. Assunta Borzacchiello

Haritha Asha
Exciton and Charge Separation : Computational Models
Tutor: Dr. Roberto Improta

Giovanna Valentino
Specialized metabolites as potential lead compounds for anticancer drug discovery
Tutor: Prof. Antonio Fiorentino

Francesca Della Sala
New strategy based on polymeric biomaterials combined to stem cells to promote pulmonary tissue regeneration
Tutor: Prof. Assunta Borzacchiello

Alessandra Monti
Molecular characterization of the interaction of PREP1 and MEIS1 with PBX1
Tutor: Dr. Nunzianna Doti

Odeta Celaj
Secondary metabolites from Mediterranean plants for nutraceutical and pharmaceutical applications
Tutor: Prof. Antonio Fiorentino

Veronica Russo
Different Impacts of MucR Binding to the babR and virB Promoters
on Gene Expression in Brucella abortus 2308
Tutor: Paolo Vincenzo Pedone

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

Jwala Priyadarsini Sivaccumar
Targeting Nodal and Cripto-1 onco-fetal proteins using recombinant
antibody fragments decorated Nanoparticles
Tutor: Menotti Ruvo and Luigi Vitagliano

Rinaldo Grazioso
Zinc finger and the metal: xenobiotic ion as structural and folding
cofactor in the prokaryotic zinc finger Ros87
Tutor: Carla Isernia

Diana Santos
Biochemical and biophysical studies of the DNA helicases DDX11 and
FANCJ involved in G-quadruplex nucleic acid metabolism
Tutor: Francesca M. Pisani

Miguel Cardoso de Azevedo Moreira
Structure and function of key macromolecules involved
in severe humane pathogeneses
Tutors: Rita Berisio - Menotti Ruvo

Molecular Cell Biology

Ana Sofia Cabaco Boavida
Exploring the roles of DDX11 and FANCJ DNA helicases in genome stability maintenance pathways
Tutor: Dr. Francesca M. Pisani

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

Alessia Casamassa
Human induced pluripotent stem cells as model to study neurodegenerative disorders: a focus on spinocerebellar ataxia type 17
Tutor: Dr. Massimo Carella; Dtt.ssa Jessica Rosati

Martina Garofalo
Molecular, biochemical and behavioural characterization of D aspartate oxidase knock-in mouse model
Tutor: Prof. Alessandro Usiello

Federica di Giacomo Russo
D-aspartic acid upregulates DAAM1 and PREP expressions during spermatogenesis
Tutor:Prof.ssa Gabriella Chieffi

Armando Di Palo
The short and the long RNAs at miR-99b/miR-let7e/miR-125a-Spaca6 locus: an open scenario in hepatocellular carcinoma
Tutor: Prof. Nicoletta Potenza

Giuseppe Petito
Effect of 3,5-diiodo-L-thyronine (T2) administration on visceral white adipose tissue inflammatory response in high-fat diet-induced overweight rats
Tutor: Antonia Lanni

Miriam Lucariello
Pharmacological targeting of the protein CtBP1/BARS in cancer and in viral infection
Tutor: Carmen Valente

Hilal Kalkan
Unravelling new pathways and innovative perspectives for treating Duchenne Muscular Dystrophy: focus on the endocannabinoid system and its interplay with the gut microbiota
Tutor: : Dr. Fabio Arturo Iannotti

Roberta Simiele
.Fasting and exercise affect T4 and BDNF signaling pathways
Tutor: Prof. Pieter de Lange

Paola Pignata
Leprel-1 is involved in angiogenesis process.
Tutor: Dr. Sandro De Falco

Giada Onorato
Identification of environmental and genetic cues that modulate neuron degeneration in C.elegans
Tutor: Dr. Elia Di Schiavi

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

Anupama Pavithran
PARP12 as a Novel Target in Cancer Resistance to Chemotherapy
Tutor: Dr. Giovanna Grimaldi

Arianna Cuomo
Physical exercise and fasting as modulators of potential biomarkers characterizing diabetes
Tutor: Prof. Pieter De Lange

Chiara Siniscalchi
miRNA role in the genotype-phenotype relationship of X chromosome aneuploidy syndromes
Tutor: Prof. Aniello Russo

Marta Mallardo
Characterization of adiponectin in cerebrovascular fluid from patients affected by Multiple Sclerosis
Tutor: Prof. Aurora Daniele

Human genetics

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

Rosita Del Prete
Generation and characterization of murine models in imprinting disorders
Tutor: Alfonso Baldi

Romina D'alterio
The role of miR-181 in Parkinson Disease
Tutor: Prof. Ssa Brunella Franco

Jamal Naderi
H3K4me3 profiles in subcutaneous pre-adipocytes of individuals with a family history of type 2 diabetes
Tutor: Dr. Claudia Miele

Laura Pignata
DNA methylation defects at multiple imprinted loci in Beckwith-Wiedemann Syndrome and/or Wilms Tumor
Tutor: Prof. Andrea Riccio

Domenico Marano
Study of the molecular interplay between MeCP2 and AUTS2 in the glycosphingolipid metabolism and its involvement in Rett syndrome pathogenesis.
Tutor: Floriana Della Ragione

Silvia Buonaiuto
A pipeline for prioritization of putatively damaging genetic variants
Tutor: Vincenza Colonna

Petrogiannakis Georgios
Identification of microRNAs involved in retinal cells degeneration and evaluation of their potential impact in the treatment of inherited retinal disorders.
Tutor: Sandro Banfi

Paola D'Ambrosio
Deep intronic mutations in the DMD gene: implications in diagnosis, phenotype and potential therapeutic approaches.
Tutor: Prof. Vincenzo Nigro

Barbara Morone
Hematopoietic differentiation of iPSCs derived from patient with the Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome into HPCs expressing CD43
Tutor: Maria R. Matarazzo

Maria Elena Onore
Linked whole genome sequencing as further step to study unsolved NMD cases
Tutor: Prof. Vincenzo Nigro

Pasqualina Cennamo
Genetic determinants of endogenous antioxidants variability in a population-based studyù
Tutor: Dr. Marina Ciullo

Session 1:

Cancer biology, Immunology, Microbiology, Drug design

Preclinical assessment of Proton Pump Inhibitors by multi slice MRI-CEST pH Imaging: A study in metastatic breast cancer

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Co-supervisor: Dr. Dario Longo, dariolivio.longo@cnr.it

PhD Cycle: 33rd Cycle

Affiliation: IBB-CNR, University of Turin.

Dysregulation of pH gradient in tumor cell is considered as a crucial hallmark of metastatic breast cancer. Magnetic Resonance Imaging – Chemical Exchange Saturation Transfer (MRI-CEST) is a novel non-invasive imaging modality to measure the extracellular pH in tumor. We proposed whether MRI-CEST approach of measuring extracellular pH can evaluate Proton Pump Inhibitors (PPIs) treatment in metastatic breast cancer. Cell viability and proliferation assays were performed in 4T1 mouse mammary carcinoma and were treated for 24 h with V-ATPase (Lansoprazole and Esomeprazole) & NHE1 (Amiloride and Cariporide) inhibitors in normoxia condition. Expression of V-ATPase and NHE1 were quantified by Western Blot and RT-PCR. Extracellular tumor pH (pHe) was measured *in cellulo* by acidification assay. 6–8 weeks old female BALB/c mice were subcutaneously injected with 4T1 mouse mammary carcinoma cells followed by PPIs treatment with V-ATPase (Lansoprazole) and NHE1 (Amiloride) inhibitors which were image by multi-slice MRI-CEST pH imaging before and after treatment. Tumor bearing mice showed a significant increase of extracellular pH post 1 and 2 weeks and reduction of tumor volume after Lansoprazole treatment. Our results suggest that non-invasive multi slice MRI-CEST pH Imaging can monitor the early response and efficacy of proton pump inhibitors in a metastatic breast cancer murine model.

Publication: Consolino L, Anemone A, Capozza M, Carella A, Irrera P, Corrado A, Dhakan C, Bracesco M, Longo DL. Non-invasive Investigation of Tumor Metabolism and Acidosis by MRI-CEST Imaging. Frontiers in Oncology. 2020;10(161).

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

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PhD cycle: 33°

Affilation: Institute of Biochemistry and Cell Biology National Research Council

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.

As results, the donor construct, containing the Antarctic Ig features and also the red fluorescent protein mCherry, as selection marker for the correct sequence insertion, has been electroporated in c-myc hybridoma cells and I have obtained about 1% mCherry positive cells.The engineered monoclonal antibody has been purified for chemical-physical and functional characterization in comparison to wild type counterpart. Overall, this "antarctized" mAb could be a promising starting point for the treatment of viral infections, since, having a greater flexibility, could assume different conformations to cope even less exposed viral antigens.

Modulating innate memory to treat inflammatory diseases

PhD student: Ana Margarida Ferreira Campos

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PhD cycle : 33° cycle

Affiliation: Institute of Biochemistry and Cell Biology National Research Council

Innate immunity displays partially-specific memory in pathogen recognition (1,2). Innate immune memory is recognized as a crucial event in mammalian host defense, being necessary to better understand the molecular mechanisms that underlie it. Recently lipids and lipid derivatives have started to be recognized as important signaling metabolites. However the information in literature regarding lipids and innate immune memory is still very limited. Among the huge diversity of lipid derivatives, glycerophosphoinositol (GroPIns), a ubiquitous bioactive compound of eukaryotic cells, displays the potential to play a central role in innate and inflammatory reactions. Studies on immune cell lines reported increased amount of GroPIns when the cells are exposed to inflammatory stimuli (3–5). Recent data points to an anti-inflammatory role of GroPIns as paracrine factor (6). Establishing how the complex lipid profile adapts, and how the levels of GPI are modulated during the development of innate immune memory, will allow better understanding on how the development of innate immune memory is regulated. Following the development of an analytical method to accurately quantify GroPIns levels, the adaptations on the complete lipid profile of human primary monocytes during the development of an *in vitro* model of innate immune memory were identified.

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Induction of anti-tumor response by filamentous bacteriophage targeting dendritic cells

Student: Roberta Manco

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PhD cycle: 35° cycle

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The delivery of stimulatory lipids and antigenic peptides derived from tumor-associated antigens (TAAs) represents a novel strategy to potentiate anti-tumor immune responses. The filamentous bacteriophage (fd) is a powerful antigen delivery system due to its intrinsic adjuvant properties. Bacteriophages can be engineered for the expression of exogenous peptides at high density on its surface, and exposed peptides can be processed and presented after bacteriophage internalization by dendritic cells (DCs), triggering strong immune responses. Cytotoxic CD8+ T-cells are required for anti-tumor immune responses and dendritic cells can prime naïve CD8+ T-cells to become efficient killer cells. Previous studies have shown the use of bacteriophages for delivering TAAs to murine DCs via specific receptor and the ability of these particles to induce antigen-specific T-cell responses. Bacteriophages can be conjugated to the immunostimulatory lipid alpha-GalactosylCeramide (α-GalCer), to activate invariant natural killer T (iNKT) cells and induce the release a wide variety of pro-inflammatory cytokines which facilitate DCs maturation and cytotoxic T cells priming. To improve antitumor response to bacteriophage delivering TAA and α-GalCer, we decide to target phage to specific cell subsets such as human DCs subpopulations or directly to tumor cells, in order to explore the activity of targeted bacteriophage in the human system.

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

PhD student: Giacomo Della Camera

Tutor: Dr. Paola Italiani (in subtitution to Dr. Diana Boraschi) italianipaola@gmail.com or paola.italiani@ibbc.cnr.it

PhD cycle: 33° cycle

Affiliation: Institute of Biochemistry and Cell Biology (IBBC) – National Research Council of Naples

Innate immunity is involved in surveillance and protection of living organisms. Innate immune cells are the first cells responsible for recognition and elimination of pathogens or foreign materials, such as nanoparticles (NPs), they come in contact with. The aim of this study is to investigate the interactions between NPs and human primary monocytes and neutrophils. In detail, we studied the activation of monocyte and neutrophils upon NPs exposure in terms of cytokine production (by ELISA) and NETosi (by fluorescence microscopy), respectively. Moreover, we investigate the capacity of NPs to induce or modulate the innate memory. The cells were stimulated with bacterial compounds (e.g., LPS, Zymosan), cytokines (e.g. IL-8) in presence or absence of NPs with different size and shape (e.g., Au, CeO2, TiO2). As first, we physical-chemically characterized NPs, with or without protein corona, in different media, and we measured nanoparticle contamination by chemical elements and endotoxin. Preliminary data suggested that, independently on the shape and size, NPs did not have a direct inflammatory effect on monocytes while they were able to modulate the innate memory. These findings may open promising perspectives for the use of NPs in immunomodulatory approaches to autoimmune and chronic inflammatory disease.

Repurposing of valproic acid and simvastatin as anticancer agents in pancreatic cancer: a new therapeutic approach in combination with chemotherapy

PhD student: Maria Rita Milone

Tutor: Alfredo Budillon (a.budillon@istitutotumori.na.it)

PhD Cycle: 33° cycle

Affiliation: Istituto Nazionale Tumori Pascale IRCCS- CROM;

Pancreatic cancer (PC) has an exceptionally high mortality rate, and few drugs made significant improvements in patient outcomes. In this regard, repurposing non-oncology already-approved drugs, might be an attractive strategy to offer more-effective treatment options easily translatable in early clinical trials. Increased expression of the mevalonate pathway and alteration of histone deacetylases (HDAC) are common aberrations found in many cancers including pancreatic cancer. We analyzed the combined antitumor effect of HDAC inhibitors plus cholesterol-lowering drugs such as statins that inhibits mevalonate pathway, on ASPC1, MDA-Panc 28, PANC1 and BXPC3 PC cell models. Significantly we showed a strong synergistic antitumor effect using valproic acid (VPA), an anticonvulsant with HDAC inhibitory activity, plus simvastatin (SIM), as assessed by calculating combination indexes and clonogenic assay on all cell lines and at very low doses, easily reached in treated patients. We confirmed the strong VPA/SIM synergism on complex tumor growth models such as cocolture of pancreatic cancer cells with fibroblasts or 3D microtissues. Furthermore we also showed a synergistic antitumor effect combining VPA/SIM plus conventional chemotherapy.
Overall these findings suggested that combining two safe generic drugs such as VPA and SIM could represent a novel therapeutic approach in combination with chemotherapy that warrants future clinical investigation in PC patients.

Characterization of the MSMEG_3762/63 efflux pump in *Mycobacterium smegmatis*

PhD student: Nicoletta Campolattano

Tutor: Prof. Lidia Muscariello (lidia.muscariello@unicampania.it)

PhD cycle: 35° cycle

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Efflux pumps are one of the known mechanisms responsible for the intrinsic resistance in *Mycobacterium tuberculosis* (*Mtb*) to antimicrobials available in clinical practices.
Our work is focused on this topic and it is based on a previous study showing up-regulation of *MSMEG_3765* in *Mycobacterium smegmatis* mc^2155 and *Rv1685c* in *M. tuberculosis* H37Rv, both encoding for a TetR-like regulator, under acid-nitrosative stress conditions (1). The MSMEG_3765 protein represses the expression of the *MSMEG_3762/63/65* operon and the orthologous region *Rv1687c/86c/85c* in *Mtb*, wherein the two last genes are annotated as components of an ABC transporter (2). To define the structural features of the efflux system MSMEG_3762/63, we performed a bioinformatic analysis on the primary aa sequence of MSMEG-3763. The most probable 3D model was generated, confirmed by different tools, and visualized using PyMol and Chimera servers. According to these findings, MSMEG-3763 forms a membrane channel and binds rifampicin and ciprofloxacin, respectively first- and second-line anti-TB drugs, as assessed by docking simulations. Moreover, the deletion mutant *M. smegmatis* (Δ*MSMEG_3763*) was isolated and a comparative analysis with the WT strain showed its involvement in biofilm formation and resistance to the above mentioned antibiotics.
Our interest is to further investigate the putative mechanisms of *MSMEG_3762/63* de-repression with putative inductors and define the possible role of the efflux system in resistance within host immune system cells and to various antimicrobial molecules.

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HSP90 identified by a proteomic approach as druggable target to reverse platinum resistance in ovarian cancer

PhD student: Rita Lombardi

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Acquired resistance to platinum-based therapies is an urgent unmet need in the management of epithelial ovarian cancer (EOC) patients. Here we characterized by an unbiased proteomics method three isogenic EOC models of acquired platinumresistance (TOV-112D, OVSAHO and MDAH-2774). Using this approach, we identified several differentially expressed proteins in platinum-resistant, compared with parental cells and the chaperone HSP90 as a central hub of these protein networks. Accordingly, up-regulation of HSP90 was observed in all platinum-resistant respect to parental cells and HSP90α knockout re-sensitizes platinum-resistant cells to cisplatin treatment. Moreover, pharmacological HSP90 inhibition using two different inhibitors (i.e. 17AAG and ganetespib) synergizes with cisplatin in killing platinum resistant cells in all tested models. Mechanistically genetic and pharmacological HSP90 inhibition induced apoptosis and increased DNA damage, particularly in platinum-resistant cells. Importantly, the antitumor activities of HSP90 inhibitors were confirmed both ex vivo in primary cultures derived from the ascites of platinum-resistant EOC patients and in vivo in EOC xenograft model. Collectively, our data suggest an innovative antitumor strategy, based on platinum-compounds plus HSP90 inhibitors, to re-challenge platinum-resistant EOC patients that might warrant further clinical evaluation.

Gold Nanoparticles contaminated by Bacterial Endotoxin: biophysical characterization, imaging and nanotoxicology

PhD student: Alessandro Verde

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PhD cycle: 34° cycle

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Gold nanoparticles (AuNPs) are nanodevice widely used in biomedical applications but their toxic effects on biological systems are not fully understood. The main goal of this study is to identify and analyse the activation of the inflammatory response associated to gold nanoparticles (AuNPs) and/or to the presence of bacterial endotoxin (or Lipopolysaccharide, LPS) on the nanoparticles' surface. To this aim, the interaction of AuNPs with LPS is analysed, the presence of LPS molecules on NPs is quantified, and the interaction of AuNPs with human primary monocytes/macrophages is investigated, in order to distinguish the intrinsic NPs biological effects from those induced by LPS.
LPS dose-dependent adsorption on 50 nm AuNPs was studied by DLS and by SERS technique in order to understand the amount of LPS that binds to NPs surface and quantify it. Internalization of bare and LPS coated 50 nm AuNPs was studied in macrophages by TEM and Raman imaging and their inflammatory effect was studied by in vitro stimulation through evaluation of inflammatory cytokine production (TNF-α).
DLS results indicate that a uniform LPS corona (8712 molecules) is formed around all NPs (2 μg) when incubated with doses greater than 500 ng, while analysis of SERS signals show a Limit of Detection (LOD) for LPS amount of the order of fg. These promising results show how SERS technique can be a reliable LPS-Sensor, while NPs imaging studies showed that NPs are localized in cytoplasmic vesicles inside macrophages. Moreover, bare NPs does not induce the production of TNF-α cytokine in treated macrophages.

Application of advanced docking strategies in the design of selective Carbonic Anhydrase IX inhibitors

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Today, the employment of various Computer Aided Drug Discovery (CADD) strategies has become essential in the different stages of the drug discovery pipeline[1]. Indeed, CADD techniques allow us to increase the efficiency and to minimize the possible failures[2]. In this context, Molecular Docking is a widely used tool that provides a snapshot of the molecular interactions between a ligand and a macromolecule. The information gathered by docking experiments can be exploited to rationally design new small molecules or improve the binding efficiency of existing ones. Our current focus is on applying an advanced docking protocol to design selective agents against the hCA (carbonic anhydrase) IX. Recently, this enzyme inhibition has been identified as a potential treatment to halt cancer progression and metastasis, especially in hypoxic cancers[3, 4]. CAs are widespread in human cells, hence the selective inhibition of the CA isoforms linked to malignancies stands as a crucial issue. Notably, CAs active site feature a catalytic zinc that is typically chelated by CAs inhibitors. To adequately describe the zinc chelation in the docking process, the cutting-edge AutoDockZn forcefield is being employed in combination with the AutoDock4.2 docking software. Thus, our efforts are now aimed at probing an in-house set of sulfonamide inhibitors with this *in silico* procedure to gain accurate insight on the requirements for hCAIX selectivity.

Keywords: CADD, Molecular docking, hypoxic cancer, AutodockZn.

References:

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Preclinical assessment of Proton Pump Inhibitors by multi slice MRI-CEST pH Imaging: A study in metastatic breast cancer

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Dysregulation of pH gradient in tumor cell is considered as a crucial hallmark of metastatic breast cancer. Magnetic Resonance Imaging – Chemical Exchange Saturation Transfer (MRI-CEST) is a novel non-invasive imaging modality to measure the extracellular pH in tumor. We proposed whether MRI-CEST approach of measuring extracellular pH can evaluate Proton Pump Inhibitors (PPIs) treatment in metastatic breast cancer. Cell viability and proliferation assays were performed in 4T1 mouse mammary carcinoma and were treated for 24 h with V-ATPase (Lansoprazole and Esomeprazole) & NHE1 (Amiloride and Cariporide) inhibitors in normoxia condition. Expression of V-ATPase and NHE1 were quantified by Western Blot and RT-PCR. Extracellular tumor pH (pHe) was measured *in cellulo* by acidification assay. 6–8 weeks old female BALB/c mice were subcutaneously injected with 4T1 mouse mammary carcinoma cells followed by PPIs treatment with V-ATPase (Lansoprazole) and NHE1 (Amiloride) inhibitors which were image by multi-slice MRI-CEST pH imaging before and after treatment. Tumor bearing mice showed a significant increase of extracellular pH post 1 and 2 weeks and reduction of tumor volume after Lansoprazole treatment. Our results suggest that non-invasive multi slice MRI-CEST pH Imaging can monitor the early response and efficacy of proton pump inhibitors in a metastatic breast cancer murine model.

Using in vivo MRI-CEST tumor pH imaging to detect early resistance to proton pump inhibitors in human prostate cancer murine models

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Two human prostate cancer cell lines (PC3 and DU145) have been treated with several proton pump inhibitors (PPIs) to assess their efficacy both *in vitro* and *in vivo*. Non-invasive *in vivo* imaging of tumour acidosis and glucose levels were used for the early detection of the therapeutic efficacy of these drugs in prostate tumor murine models. Cell viability and pH measurements were performed *in vitro* for the following drugs: lansoprazole, esomeprazole (V-ATPases), cariporide and amiloride (NHE1). For *in vivo* experiments, athymic nude mice were inoculated subcutaneously or orthotopically with human prostate PC3 or DU145 cells, treated with esomeprazole (dose: 2.5 mg/kg) for two weeks and tumor growth was monitored. MRI-CEST tumor pH imaging and FDG-PET were perfomed one week after treatment for assessing early treatment response by measuring tumor acidosis and glucose uptake.

Despite a potent effect of esomeprazole *in vitro*, *in vivo* studies showed no reduction in tumor growth for both the two prostate tumor murine models after two weeks of treatment. Interestingly, tumor pH imaging proved no differences in tumor acidosis between treated and control mice that were confirmed by the FDG-PET approach.

Esomeprazole provided a marked pH alteration *in vitro* but the inability to change tumor acidosis in vivo, anticipated the in vivo inefficacy to reduce tumor growth. Therefore, this approach can be used to detect early response and upfront resistance to PPIs.

Search for orphan drugs using bioinformatics tool and *in vitro* validation

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The present work is focused on the identification of drugs for the treatment of disorders caused by protein instability (1). By several approaches we can test the effect of repurposed or new compounds, as in the case of TTR- amyloidosis or PMM2- congenital disorder of glycosylation (CDG).
Mutations in the gene encoding transthyretin (TTR) promote tetramer dissociation that underlie fibril formation. Iodinated 4,4'-bipyridines were characterized as TTR stabilizers in collaboration with Dr. Peluso who synthesized the molecules. After purifying the protein, I assessed the compounds activity through a turbidity assay. Three compounds, named (M)-8, (M)-9 and (P)-9, resulted effective in reducing fibril formation to 16- 40% for wild type and 40- 50% for mutants (2).
The most frequent glycosylation defect is caused by mutations in phosphomannomutase2 (PMM2). The defective enzyme (transforming mannose 6-phosphate into mannose 1-phosphate) leads to hypoglycosylation of numerous glycoproteins. Looking for pharmacological chaperons to enhance PMM2 stability, some molecules were already identified (i.e. βG16) (3). Moreover, it was conceived a strategy to promote the transport of such molecules through the cell membrane. G16 was chemically modified by Dr. Rimoli obtaining lipophilic G16 used to treat PMM2- CDG cells. A rescue of the PMM2 activity was reported (preliminary results).

Effect of non-psychotropic cannabinoids on androgen deprivation therapy in high-fat diet- induced prostate cancer: implications for gut microbiome

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Prostate cancer (PCa) is the second most frequent cause of tumor-associated mortality worldwide. Tumour growth depends on androgens, therefore, the mainstay treatment is based on androgen deprivation therapy (ADT). High fat diet (HFD) promotes prostate tumour carcinogenesis via a dietary-induced inflammation that contributes to negative prognosis, but the underlying mechanisms are still poorly understood. Several studies consider certain phytocannabinoids promising anti-cancer agents and controllers of food intake, fat browning, and lipid metabolism.

We investigated the effect of phytocannabinoids (CBD + CBG) alone and combined with anti-androgen enzalutamide on tumour progression in transgenic adenocarcinoma mouse prostate model (TRAMP) mice under HFD and regular diet (RD). Histopathological analysis and gut microbiota profiling showed that HFD exacerbated PCa development and induced gut microbiota dysbiosis lowering abundance of bacterial phylum Bacteroidetes, a condition that can upgrade inflammatory cytokines. Single treatments with anti-androgen or phytocannabinoids significantly inhibited tumour development only in RD-fed mice. Combined treatments were effective in mice under both diet regimens. In HFD-fed mice combined treatment reverted the lowered levels of Bacteroidetes-related bacteria.

The study reveals that phytocannabinoids combined with ADT drug improves inhibition of tumor growth in HFD-induced prostate cancer and modifies gut microbiota composition. These findings indicate a novel, more beneficial in respect to monotherapy, therapeutic approach for obesity-induced PCa.

Effect of Ruta graveolens water extract on ischemic damage and neurological deficits in a rat model of transient focal brain ischemia

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Introduction and objectives Current therapeutic approaches of ischemic stroke are based on thrombolytic drugs which have a narrow therapeutic window and important side effects. Therefore, research for new approaches is necessary.
Natural compounds are gaining more attention in recent years in a one drug multi target paradigm.
In our study, we set up a brain ischemic model in rats through the transient occlusion of Middle Cerebral Artery (MCA) by an intraluminal filament to evaluate the neuroprotective potential of the aqueous extract from leaves of *Ruta graveolens* (RGWE) in brain ischemia.
Results. Our preliminary data, based on a small number of animals (n=3 per experimental group) show that ip injection of two different doses of RGWE, containing respectively 10 and 30 mg/kg of *Rutin*, the main component of the extract, may have a neuroprotective effect on ischemic volume. Neurological deficits measured 24 after stroke induction show that the low dose (10 mg/kg) has a protective effect.
Conclusions. Natural compounds may have a neuroprotective effect on ischemic volume and on neurological deficits.
In the future experiments it will be important to increase the number of animals per experimental group and to define a reliable time window.

Effect of non-psychotropic cannabinoids on tumour growth in hormone refractory prostate cancer

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Cancer cells follow a unique metabolic programming by preferring aerobic glycolysis as firstly observed by Otto Warburg in 1956. Cancerous phenotype is essentially backed by genetic mutations triggering several oncogenic signaling pathways that rewire the cellular metabolism to meet the highly bioenergetic and biomass requirements of proliferating cells. This crosstalk, although not completely understood yet, is a potential target for new promising interventions against cancer.

We previously demonstrated that CBD, alone or in combination with CBG, significantly reduced ($p<0.05$ and $p<0.001$, respectively) tumour progression in TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP). We set up *in vivo* and *in vitro* models of hormone refractory prostate cancer using enzalutamide, an androgen receptor antagonist. Combined treatment with CBD and CBG (1:1) significantly reduced tumour relapse ($p=0.0052$).

We investigated how purified plant cannabinoids (CBD and CBG) affect the favourite metabolic system of this malignant tumour form. CBD showed more potency than CBG, as it up-regulates glycolysis and inhibits oxidative phosphorylation in enzalutamide-resistant cells. These metabolic changes lead also to notable shifts of specific oncogenic related signaling pathways in these cells (i.e. HIF-1a, BNIP3, PTEN and AMPK/ULK-1). The study supports the clinical testing of phytocannabinoids as metabolic intervention and adjuvant therapy on HRPC.

Hybrid lipid/polymer nanoparticles for pulmonary delivery of siRNA in cystic fibrosis lung inflammation

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The down-regulation of genes directly involved in the pathogenesis of severe lung diseases through pulmonary delivery of short RNA fragments, also known as siRNA, has gained recently remarkable research interest, especially in cystic fibrosis (CF). In this context, the general aim of this work is the development of inhalable hybrid nanoparticles (hNPs) for siRNA delivery made up of a combination of lipids and polymers. To this purpose, the therapeutic potential of hNPs delivering a siRNA pool against one of the most critical signals in evoking the inflammatory response in CF, the nuclear factor-κB (NF-κB), is under investigation. hNPs loaded with siRNA against NF-κB were prepared from poly(lactic-co-glycolic acid) and endogenous phospholipids (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine -DPPC- and 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol) -DSPE-PEG-). The most adequate formulation conditions to produce non-PEGylated and PEGylated siRNA-loaded hNPs with optimal aerosolization and mucus-penetrating properties have been identified. *In vitro* data suggest that siRNA-loaded hNPs are not cytotoxic and may penetrate lung extracellular barriers, allowing siRNA uptake inside human bronchial epithelial cells. In perspective, a validated rat model of lung inflammation will be employed in the *in vivo* efficacy studies of the optimized siRNA-loaded hNPs.

Isolation and characterization of PAH's degradaing bacteria by *Dietzia* and *Rhodococcus* sp.

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The increasing chemical and biological pressure, conjointly induced by human activities into the ecosystem, had created adverse effect on public health and environment. Among others, polycyclic aromatic hydrocarbons (PAH's) is of major concern due to their toxic, genotoxic, mutagenic properties. Microorganisms has evolved numerous metabolic strategies in assimilating hydrocarbons as sole carbon source and converting them into non-toxic products. Two bacteria of genus viz., *Dietzia, Rhodococcus* (identified by 16s rRNA sequencing) isolated from various marine sediments have demonstrated the ability to grow on wide range of PAHs. Pyrene and phenanthrene was used as model PAH's to study the degradative ability of these two genera. With help of Gas Chromatography-Mass Spectrometry (GC-MS), percentage of degradation of hydrocarbons were obtained. The whole genome sequencing data of *Dietzia* and *Rhodococcous* reveals the presence of ring hydroxylating dioxygenases and monooxygenases enzymes making them the suitable candidates for bioremediation. The work involves a combinatorial approach of analytical and omics approach in unravelling the pathways involved in hydrocarbon degradation. These results help us in choosing the suitable and efficient microorganisms for bioremediation for dynamic polluted environments.

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 becoming more evident factors in cancer development and progression.
In our laboratory, we recently discovered that the Golgi localized onco-protein GOLPH3, that is often amplified in solid tumours exerts its oncogenic activity through regulation of Golgi glycosyltransferases. Specifically, we found that GOLPH3 positively regulates glycosphingolipid (GSL) synthesis by controlling the localization and proteolysis of B4GALT5/LCS, a Golgi glycosyltransferase. Cell specific changes in sphingolipid metabolism induced by the manipulation of GOLPH3 levels has an impact on receptor tyrosine kinases (RTKs) sensitivity towards their ligands, thus influencing cell growth.
Interestingly, we found that B4GALT5 which is necessary and sufficient for the oncogenic effect of GOLPH3, is amplified in same tumor type but in different patients (GOLPH3 and B4GALT5 amplification are mutually exclusive). Thus, we reasoned if B4GALT5 is an oncogene? If so, what is the molecular mechanism of its oncogenesis?
Gain-of-function experiments in immortalized NIH3T3 showed that B4GALT5 promotes oncogenic transformation, cell growth and proliferation. Genetic ablation in B4GALT5 amplified cancer cell lines showed marked sensitivity to growth, mitogenic signals and colony formation. Mechanistically, B4GALT5 specifically alters Globoside (Gb3Cer or Gb4Cer) biosynthesis thus, affecting 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 cancers. 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 aptamer-based therapeutics to selectively target the stem-like cancer cells

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The stem-like phenotype in cancer is the result of epigenetic and genetic alterations leading to the expression of stemness-related genes. In this view, their targeting could lead to the development of clinically relevant strategies for cancer therapy. New promising molecules are aptamers, short synthetic nucleic acids selected for specific binding to target of interest.

The aim of this work is to address the stem cell cancer phenotype targeting by developing a novel aptamer against **SALL4**, a transcription factor essential for the maintenance of pluripotency and self-renewal capacity of ESCs, whose expression is found to be de-regulated and aberrantly expressed in various cancers. It has been demonstrated that SALL4 acts as a transcription repressor of tumor suppressor gene PTEN by interacting with the histone deacetylase (HDAC) complex NURD and that blocking SALL4-NuRD interaction hampers its repressive function reversing the aggressive phenotype. Base on this information, two types of aptamer selection based on applying protein-SELEX (Systematic Evolution of Ligands by EXponential enrichment) were developed: a more generic one, in which aptamers against SALL4 were selected; and an epitope specific one, in which aptamers were selected to specifically disrupt the interaction. Finally, the selected sequence was shortened and further characterized for its binding to SALL4.

Identification of lead compounds from Marine Natural Products as novel therapeutic strategies to treat neurodegenerative disorders

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Neurological disorders constitute over the 6% of the global burden disease which common feature is the impairment of immune response, in particular of microglia cells (1). Triggering receptor expressed on myeloid cells 2 (TREM2) is emerging as an important immunomodulatory receptor with a key role in modulating microglial metabolic fitness, and enabling microglial activation, migration and phagocytosis. Herein, the precise activation and/or inhibition of TREM2 could modulate the neuro-inflammatory process in neuropathological conditions like Alzheimer disease or multiple sclerosis (2-3).
Our studies are aimed to screen several natural compounds targeting specifically TREM2, as, given their wide biodiversity, small molecules isolated from marine micro- and macro-organisms have been shown to have relevant biological properties and extremely significant therapeutic uses. However, crude extracts of marine organisms contain high percentage of salts which are toxic for many cells and impair the recovery of unknown metabolites. Therefore, we prepared a drug discovery library of about four-hundred raw fractions starting from different species of marine organisms, of which sponges, diatoms, algae, bryozoans, tunicates and molluscs, applying a novel method on Solid Phase Extraction (SPE) (4). All those fractions were tested, firstly, on TREM2 reporter cell line. After the screening, we selected the most active and less toxic down to twenty promising fractions, which were mainly from sponges and algae. To optimize the selection outwards the discovery of a specific ligand for TREM2, those fractions were tested on reporter cell lines expressing other receptors that play a key role in the innate immune system, such as Dectin1, Toll-like receptors (TLR) 4 and TLR 2, which are known to be key regulatory components of inflammation. Surprisingly, out of twenty only four were active for TLRs or Dectin1., Three active fractions of two marine organisms were selected for a further in-depth investigation. Starting with a scale-up of the original material, we evaluated the reproducibility of the fractionation obtaining an almost identical pattern of activity. Thus, we proceeded with further purification of the selected active SPE-fractions. Each sub-fractions were retested on all the reporter cell lines above mentioned in order to identify the molecules or the family of compounds responsible for the activity. Whit this approach, we identified a new potential class of molecules which could potentially bind TREM2. Afterwards, the effectiveness in activating specifically TREM2 and the mechanism of action of lead compounds will be further envisaged using microglial cell lines.

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Novel antimicrobial biosurfactants from Antarctica

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Biosurfactants are surface-active agents that have gained a large biotechnological interest in the last years because they have a lot of field of application, such as bioremediation, food industry, biomedicine, agriculture, cosmetic and detergent industries. We isolated from sediments collected in Edmonson Point, Antarctica, a strain identified as *Pseudomonas gessardii* able to produce biosurfactants. LC-HRMS and LC-MS/MS analysis revealed the presence of 17 different mono-rhamnolipids in the extract of which six, Rha-C12:1-C8, Rha-C12:1-C12:1, Rha-C14:1-C12:1, Rha-C16:1-C10, Rha-C14:1-C12, and Rha-C12-C14:1, were never described before. Successively, the antimicrobial potential of this mixture was evaluated towards a panel of human pathogens, obtaining interesting results against *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* and reporting for the first-time antimicrobial activity of rhamnolipids towards *Stenotrophomonas maltophilia*. We also correlated the increments of the antimicrobial activity to Rha-C12:1-C12:1, Rha-C14:1-C10 and Rha-C16:1-C10, through their relative abundance in the RL mixtures.

Furthermore, a different class of biosurfactants, trehalolipids, are now under investigation. These molecules are produced by a *Rhodococcus spp.* isolated by our group from marine samples near Deception Island, Antarctica. Preliminary LC-MS and LC-MS/MS analyses suggested that this strain is able to produce new trehalolipids, but further analyses are required to confirm the data.

Marine environment as a source of new multiactivity pigments and bioactive molecules

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The marine environment is among the most diverse habitats on the Earth, it shows singular environmental conditions which stimulate the organisms that inhabit this habitat to activate unusual metabolic pathways. Our team isolated several bacteria of diverse taxa from sediments coming from all over the world. An interesting bacterium was isolated from sediments coming from Ria Formosa's lagoon, Portugal, it was identified to be a *Vibrio sp.,* showing high 16S rRNA gene sequencing similarity with *Vibrio spartinae*. Spectrophotometric and HPLC-MS analysis revealed the presence of prodigiosin and cycloprodigiosin as major metabolites, followed by several peaks due to different prodigiosin-like molecules. Among them, one new prodigiosin derivatives named isoheptylprodigiosin was isolated and subjected to a in deep bioactivity-screening.

A different bacterial isolation at different pH values was conducted on sponge samples coming from the volcanic environment of Ischia island, known to be characterized by the presence of underwater CO_2 vents. This study raised the presence of an interesting strain belonging to *Pantoea* genus, this bacterium was subjected to a complete metabolic study in different conditions through a combination of OSMAC and Molecular Networking approaches, highlighting remarkable differences in the metabolome due to the use of inorganic nitrogen.

Identification and characterization of *Gm4340 (Thoc4)* gene expression in mRNA Export in Embryonic Stem Cells

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TRanscription and EXport (TREX) is a conserved multisubunit complex, also known as THO complex essential for ESCs self-renewal and differentiation throughout life. By linking transcription, mRNA processing and export together, the classical approach plays a vital role in the gene expression pathway. Besides, this complex prevents DNA damage and regulates the cell cycle. *Gm4340 (Thoc4)* is a member of this complex associated with Kaposi Sarcoma and Esophageal Diverticulosis. To date, there is not much information is available related to *Gm4340 (THOC4)* and mRNA Translocation. In this context, we aim to fill an essential gap in the post-transcription regulatory mechanisms by identifying functional pathways involved in this critical but previously not studied period. **Methods**: wild type mouse blastocyst-derived ES cell lines were obtained, propagated, and maintain to optimize transgene expression in ES cells; we have generated P2lox-Gm4340-GFP expressing construct under the control of CAG promoter. **Anticipated Results:** Initially, we identified gm4340 is highly expressed in a 2-cell state and based on the literature we found that it plays a key role in the transport of mRNA. We are anticipating the result of how efficient this gene is working in our system and the role of gm4340 in embryonic development.

Targeting CA IX prevents hypoxia-induced cell plasticity in Triple Negative Breast Cancer

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Background: Cell plasticity is the ability that cells have to modify their phenotype adapting to the environment. The cancer progression is under strict control of the tumor microenvironment that strongly determinates its successful occurrence by regulating the behavioral changes of tumor cells. The cross-talk between cancer and stromal cells, the interactions with extracellular matrix, hypoxia and acidosis contribute to trigger a new tumor cell identity and to enhance tumors heterogeneity and metastatic spread. In highly aggressive triple-negative breast cancer (TNBC), tumor cells show a great capability to change their phenotype under the pressure of hypoxic microenvironment. In this study, we investigated whether targeting the hypoxia-induced protein carbonic anhydrase IX (CA IX), could reduce TNBC cell phenotypic switching involved in processes associated with poor prognosis such as vascular mimicry (VM) and cancer stem cells (CSCs).
Methods: To explore whether CAIX levels could be associated with TNBC, we analyzed its expression in a public data-set of 198 TNBC samples as well as in MDA-MB-231 and BT-549 TNBC cell lines. All experiments were performed by growing the cells in normoxic (21% O_2) and hypoxic conditions (1% O_2) and silencing CAIX expression using a specific siRNA. Cell migration and invasion assays were carried out using Boyden chamber uncoated and coated with matrigel, respectively. TNBC cell capability to form VM and mammospheres with stemness features were analyzed growing cells on Matrigel and Vitrogel-RGD, respectively. Furthermore, the expression of HIF-1 ✔, CAIX and mesenchymal markers were analyzed by western blotting. In addition, the effect of a novel CAIX (RC44) inhibitor on TNBC cell plasticity was tested.
Results: The treatment of TNBC cell lines with a specific CA IX siRNA or with a specific small-molecule inhibitor (RC44) severely impaired their ability to form VM and mammospheres as well as reduced their metastatic potential. In addition, RC44 inhibitor was able to hamper the signal pathways involved in triggering VM and CSC formation.
Conclusion: These results demonstrate that targeting hypoxia-induced cell plasticity through CA IX inhibition could be a new opportunity to selectively reduce VM and CSCs thus improving the efficiency of existing therapies in TNBC.

Study of HLA-DQA1 and DQB1 risk alleles conferring genetic susceptibility to Celiac Disease

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Celiac disease (CD), a multifactorial and polygenic disorder caused by an autoimmune response to the ingestion of gluten, has a strong genetic association with Human Leucocytes Antigen (HLA). The expression of HLA class II predisposing genes by Antigen Presenting Cells (APC) contributes to the anti-gluten $CD4^+$ T cells immune response; we found that the expression of risk alleles is much higher than the expression of non-CD-associated alleles with significant differences between APC from celiac patients respect to healthy subjects. The expression of HLA-DQA1 and DQB1 transcripts was also evaluated in macrophages of CD patients in gluten free diet and healthy controls in order to investigate the function of these cells as APC in CD remission. We are now exploring the expression of the HLA-DQB1-AS1 antisense RNA to unravel its function on the DQB1*02 risk allele regulation. In addition, performing RNA sequencing analysis, many up- or down-regulated genes were identified in CD patients; these genes could have a significant role in the pathogenesis and in the onset of CD. These findings could provide new insight in the proposal of risk allele expression as a possible approach for a personalized diagnosis and for risk disease stratification.

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 by the development and use of an innovative platform of drug discovery. A drug screening is performed using 59 different marine samples.
The key element of the drug discovery platform consists in desalting and fractionate the crude extracts using a solid phase extraction procedure, allowing to yield samples with lower complexity and can be tested with higher sensitivity.
The crude extracts together with their fractions are next tested for identifying biological activities. The biological activities of interest in this project are cytotoxicity, antibiotic activity, and inhibition of the PTP1B enzyme, a therapeutic target of diabetes type 2.
For the cytotoxicity test, this procedure allowed to identify activity in 46 organisms (versus 21 testing only the extracts). As regards the antibiotic activity, we identified 26 active organisms (versus only 3 testing only the extracts). Finally, for diabetes, 4 organisms were identified, all of them undetectable testing the mere extracts.
Three promising fractions were identified as promising and an iterative bioassay-guided fractionation process started, in order to identify the active, ideally novel, chemical entities.

Regulation of HLA DQ2.5 genes in subjects with high/moderate risk to develop Celiac Disease

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The genetic susceptibility to develop CD is mainly associated with HLA-DQA1*05 and HLA-DQB1*02 class II genes encoding for DQ2.5 molecules. The DQ2.5 heterodimer is expressed on antigen presenting cells (APCs) and presents gluten peptides to $CD4^+$ T cells that orchestrate the inflammatory cascade leading to the intestinal mucosa damage typical of CD. This project is based on recent findings demonstrating that the strength of pathogenic $CD4^+$ T cell response to dietary gluten is primarily determined by the expression of HLA class II CD risk alleles in professional APCs, and not by their genotype.

The main aims are:

1. **Measurement** of expression of DQA1*05 and HLA-DQB1*02 risk alleles respect to non-CD associated alleles in APC carrying different heterozygous genotypes with high or moderate CD risk respect to healthy subjects.

2. **Molecular analysis** aiming to identify the mechanisms responsible for the differential expression of risk alleles. The purpose is to investigate the long-range transcriptional regulation of DQA1*05 and DQB1*02 alleles, in order to identify enhancer and/or distal promoter sequences in the intergenic regions.

3. **Design an innovative device** for the measurement of CD risk alleles expression. The results will provide a tool for the early diagnosis of celiac family members, monitoring of clinical remission and stratification the disease risk.

Session 2:

Gene Regulation and Computational Biology

Investigation on regulatory components and differentially methylated regions associated with genomic imprinting and imprinting disorders

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Genomic imprinting is an epigenetic phenomenon that involves selective expression of some genes known as imprinted genes (IGs) from one of the parental alleles. This allele-specific expression ensures proper development of organisms during early stages, failure of which results in genomic imprinting disorders. The integrity of genomic imprinting is maintained by a specific group of proteins, which occupy regulatory sites called imprinting control regions (ICRs). ICRs are differentially methylated regions (DMRs), which can control the expression of IGs depending upon its methylation state. Identification of allele-specific modulators, differential methylation profile at ICRs and regulation of allele-specific IGs expression has recently emerged as potential aspects in further exploring the basis of genomic imprinting.

In our study using mouse embryonic stem cells (mESCs), we identified two transcription factors binding at maternally methylated ICRs, importance of which yet need to be studied. In extension to our previous study on expression of imprinted genes in mESCs, we differentiated mESCs to neural progenitors lineage in order to assess the changes in methylation at ICRs and monoallelic expression of imprinted genes upon cellular differentiation. Furthermore, we have investigated the methylation profile at DMRs in human patients who are clinically suspected or diagnosed with imprinting disorders like Beckwith-Weidemann syndrome and hydatidiform mole.

Our studies have thus allowed us to gain insights into genomic imprinting and add some missing knowledge in research on genomic imprinting. In addition to this, our findings on human subjects may help in molecular diagnosis of imprinting disorders.

New statistical methodologies for Omic Network Integration

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Nowadays, the massively parallel sequencing technology, known as NGS, has revolutionized the biological sciences. Even though there is a massive amount of data available, the difficulty comes with extracting meaningful information. My research activity is to develop new computational methods that integrate different omics types to give a global view of the actors who play a fundamental role in the onset and progression of a given disease or a biological process. In recent years, omics integration has begun through different methodologies such as dimensionality reduction (Argelaguet, et al. 2018) and network similarity analysis (Wang et al. 2014). We are trying to integrate omics data using network approaches due to their versatility. There are different types of biological networks such as protein-protein interaction network, gene regulatory network, gene co-expression, and co-methylation network. We started studying the Gene Expression data from patients with BRCA in different stages. We aim to identify general breast cancer information by building a consensus network and then obtaining stage-specific networks. On the network thus obtained, we will add the DNA Methylation data to expand the knowledge about cancer. The methodology we are conceiving can be extended to different types of cancers.

Network similarity measures for tumor metabolic networks

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Network modelling of cellular and molecular interactions has evolved into a powerful tool due to the vast amount of data collected with omics technologies. Network comparison methods enable us to study the extent of similarities/dissimilarities between cells/tissues and also those between healthy and diseased conditions.

My PhD research involves the use of probability distribution-based network distances for the analysis of tumor metabolic networks. The networks are constructed by integrating tissue-specific genome-scale metabolic models and gene expression data. Each network is represented using its node distance distribution and transition probability matrices. Computation of pairwise distances between these distribution matrices results in a Gram matrix which is then used in learning tasks.

In [1], metabolic networks were constructed for lung, kidney and breast cancer patient samples from the TCGA database. Classification performed on the network distance matrices, showed that the classification accuracies were comparable to those obtained with gene expression data.

As these networks contain thousands of nodes and edges, we explored network simplification approaches to reduce computational times. In [2], network simplification was performed by selecting nodes with high eigen centrality. We show that the classification results on these simplified networks were equivalent to the whole networks, along with a significant reduction in time. In [3], we developed a computational workflow for network clustering, which involves graph summarization and clustering of the inter-network distances of the summarized graphs.

We studied the role of alternative splicing in single cells derived from breast cancer samples for determining tumor heterogeneity in [4]. Our results show the involvement of transcriptional regulation by the splicing machinery in determining tumor heterogeneity. We demonstrated that isoforms expression and splicing event prediction, at the single-cell level, provide useful insights to discriminate the tumor subtypes better. In addition to the splicing analysis, we built metabolic networks for individual tumor cells and performed network analysis to study heterogeneity based on metabolic activity.

Bioinformatic analysis of RNA-seq data to identify molecular pathways involved in two imprinting disorders: Beckwith-Wiedemann and Silver-Russell syndromes

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In humans, there are approximately 100 genes that are expressed in a monoallelic and parent-origin-specific manner. These are called imprinted genes. Imprinted genes are organized in clusters that are called imprinted domains; each of them is controlled by an Imprinted Control Region (ICR) that show allele-specific DNA methylation and histone marks essential to control the imprinted expression of the flanking genes. Alteration of the expression of imprinted genes leads to imprinting disorders. Dysregulation of the H19/IGF2 imprinted locus (IC1) in chromosome 11p15.5 is associated with two fetal growth disorders: Beckwith-Wiedemann syndrome (BWS) that shows overgrowth and Silver-Russell syndrome (SRS) that shows growth retardation.

In order to investigate the molecular mechanisms altered in the two imprinting disorders we have performed RNA sequencing (RNA-seq) from a previously generated mouse line modeling for BWS and SRS, also we downloaded from Gene Expression Omnibus (NCBI-GEO), public repositories, RNA-seq datasets from mice with an alteration in the imprinted 11p15.5 region. Using a bioinformatic pipeline we identified Differential Expressed Genes (DEgs) compared with wild type mice that are involved in several molecular pathways.

Through this genomic and computational approach, we could identify genes and molecular pathways that are associated with the phenotype of both imprinting disorders.

The role of NLRP5 in genomic imprinting. A new mutant mouse model

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In mammals, a subset of genes is characterized by a monoallelic and parent-of-origin-dependent expression, with a fundamental role in the embryo-development. This phenomenon is called genomic imprinting. It is regulated by epigenetic mechanisms as DNA methylation and histone modifications. A subset of patients affected by imprinting disorders is characterized by multi-locus imprinting disturbances (MLID). Loss-of-function mutations affecting components of the sub-cortical maternal complex (SCMC) have been found in healthy women with reproductive problems and/or offspring with variable imprinting disorders. The mechanisms by which SCMC variants result in DNA methylation abnormalities is unknown. We generated a mouse with a hypomorphic missense variant, in the PADI6 gene. We chose a variant that was previously identified in compound heterozygosity together with a truncating mutation in the mother of two siblings affected by Beckwith-Wiedemenn syndrome and MLID. The variant was introduced into mouse ES cells by homologous recombination, and transgenic mice were generated by blastocyst injection. The fertility of the homozygous female mice will be investigated. DNA methylation will be analyzed genome-wide in the oocytes of the homozygous female mice and in their progeny. We believe this approach may help understanding the mechanism by which Padi6, and in general SCMC genes, affect genomic imprinting.

Identification of the gene networks that modulate ABCA4 expression

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Stargardt disease is an inherited retinal disorder caused by biallelic mutations in the *ABCA4* gene. To gain insight into the regulation of *ABCA4* expression, we decided to analyse already available RNAseq datasets. I retrieved bulk RNAseq human retina data from 161 non-visually impaired post-mortem donors from two different datasets. Subsequent analysis allowed me to predict the presence of more than 60 putative novel transcripts in *ABCA4* (Observed transcriptome (ObsT)). I sought to determine whether the newly predicted transcripts could harbour known intronic pathogenic variants previously annotated but I did not find significant results.

I then aimed at reconstructing the gene networks underlying the expression of *ABCA4* as well as of other biologically relevant retinal genes by co-expression analysis. The analysis, still in progress, allowed me to identify more than 15 co-expression gene clusters per dataset. I am now analysing the following gene clusters: a) the one containing the *ABCA4* gene; b) those enriched in photoreceptor-expressed genes; c) those enriched in Inherited Retinal Disease genes; d) those enriched in retina-specific transcription factor genes; and e) those enriched in selected long noncoding RNAs. I am currently looking at the composition of these co-expression clusters and performing Gene Ontology and KEGG analyses to predict their putative function.

Integrated analysis of epigenomic and trancriptomic signature in patient derived iPSCs carrying DNMT3B mutations and their isogenic lines with restored DNMT3B following CRISPR/Cas9

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DNMT3B is one of the major de novo methyltransferases responsible for the genome-wide methylation during the early stages of embryonic development. Immunodeficiency, Centromeric instability and Facial anomalies syndrome (ICF syndrome) is a rare autosomal recessive disorder where about 60% of the patients carry hypomorphic mutations in DNMT3B gene. The wide spectrum and varying degree of severity of clinical phenotypes can be postulated to the genome-wide effect of DNMT3B dysfunction. To elucidate the early molecular mechanisms involved in the pathogenesis of ICF syndrome , we have performed comparative analysis of Whole Genome Bisulfite Sequencing (WGBS), and RNA-Seq datasets from control, patient-derived iPSCs and their CRISPR/Cas9-corrected clones.

The global level of DNA methylation was not dramatically reduced in patients compared to controls. However, we identified about 27,000 differentially methylated regions (DMRs) uniformly distributed across the chromosomes. Approximately 74% of DMR that were hypomethylated in patients were rescued in both of their respective corrected clones. A significant percentage of differentially expressed genes in patients compared to controls were associated to DMRs.

To understand the complex interplay between the methylation and expression defects within the chromatin context, ChIP-Seq data for DNMT3B binding and H3K4me3 and H3K36me3 marks were analysed and the integrated results will be discussed in detail.

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. Now we successfully bred XMecp2:mCherry mice with 94% C57/B6 background and waiting for the XMecp2:eGFP mice sending from Adrian Bird Lab for double knock in mice.

Session 3:

Structure and Functions of Biomolecules

Determination of primary structure, gene organization and antifungal activity of Ageritin, the first ribotoxin-like protein from edible mushrooms

PhD Days 2020

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The edible mushroom *Agrocybe aegerita* produces a ribotoxin-like protein known as Ageritin. The protein is basic (pI≥9.5) and consists of 135 amino acid residues (~15-kDa) with a high thermal stability (Tm=78°C), a single free cysteinyl residue and α/β fold by homology modeling [1, 2]. This toxin is able to release the α-fragment when incubated with ribosomes from several sources [3] and exerts: i) cytotoxicity [4, 5]; ii) antifungal activity [4]; and iii) insecticidal activity [6].
During the last PhD year, *ageritin*-gene was characterized by sequence analysis. It contains typical fungal genes features, such as three short introns (60-55 and 69 bp) at the 5' region of the coding sequence and typical splice junctions. It codes for a precursor of 156 amino acids (~17-kDa) containing an additional N-terminal peptide of 21 amino acid residues, absent in the purified toxin, as confirmed also by Western blot analysis.
Finally, the immunolocalization by CLSM and TEM proves that Ageritin has vacuolar localization in hyphae. These data coupled with a bioinformatics approach suggested that the N-terminal peptide of Ageritin is a new signal peptide in fungi involved in intracellular routing from endoplasmic reticulum to vacuole, necessary for self-defence of *A. aegerita* ribosomes from Ageritin toxicity.

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Secretome analysis reveals a role of pentraxin-3 in modulating the angiogenic activity of high mobility group box 1 in breast cancer

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Cell secretome is considered a valuable reservoir of potential biomarkers and/or therapeutic targets for cancer and other diseases. Among cancer biomarkers, long pentraxin 3 (PTX3) may holds great promise for potential therapeutic and prognostic implications. It has been reported that PTX3 inhibits the growth and the vascularization of FGF-dependent tumour. However, whether PTX3 acts as a good or bad cop in cancer still remains unclear for its dual role in inhibiting and promoting tumour progression. In particular, the role of PTX3 in triple-negative breast cancers has never been investigated yet.

Secretome analysis from conditioned media of cultured tumour cells could offer a comprehensive source of relevant molecular information to better understand cancer pathophysiology. During the last decade, mass spectrometry-based proteomic approaches proved to be a powerful tool to identify and quantify secreted proteins.

In this project, a quantitative high resolution nano-LC-MS/MS TMT isobaric labeling-based approach has been applied to the characterisation of the molecular changes occurring in MDA MB 468 secretome, with respect to control cell line, following PTX3 over-expression.

By this strategy, the molecular hallmarks involved in the increased aggressiveness of MDA-MB 468 cells overexpressing PTX3 have been delineated and high mobility group box 1 (HMGB1) has been identified as a key factor capable of inducing angiogenesis.

Structural Analysis of Metal ion binding Human Prion Proteins using NMR Methodologies

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Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful tool for biologists and chemists to study the structure and interaction of biological molecules [1]. This project aims to analyse the function of cellular prion proteins and its interactions with copper ions to study how this metal binding can influence protein and its function [2]. Prion diseases are a group of neurodegenerative diseases in which normal cellular prion protein PrP^{C} into the infectious form PrP^{Sc}. However the function of PrP^{c} is still unknown, it is necessary to determine the interaction between PrP^{c} with copper ions [3]. Recent studies shows that prion protein is highly affinity towards copper binding [4]. In our studies we used NMR techniques to analyse role of metal ion in prion conversion and with a special focus on its binding site. During our studies we found out that prion proteins contain sugar molecules over their structure, this shows glycosylation expressed by neurones and other cells. The presence of sugar molecules forms highly diverse in protein structure and more conserved. The copper binding sugar molecule containing PrP^{c} exhibits superoxide dimutase activity. By understanding this process can help us to learn more about prion diseases. Detailed study on conformational equilibria was studied using NMR.

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PTFE nanoplastics impact on Bifidobacterium growth and biofilm formation

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Micro-nanoplastics as environmental pollutants have received in the last few years a wide attention from the scientific community regarding the risks to animal and human health. We focused at first our attention on the effects on PTFE nanoplastics on the growth and biofilm formation by *Bifidobacterium (Bifidobacterium breve* in our experiments*)*, a genus that included gut commensals with reported health-promoting activities.

Experiments were performed on polystyrene plates for which it has been demonstrated *Bifidobacterium* capability to form biofilm. We observed that the presence of nanoplastic reduced biofilm formation on polystyrene. Moreover, we have found differences on *Bifidobacterium* growth, apparently better in the presence of nanoplastic, possibly due to the bacterium partial inability to form biofilm: data on growth in presence and in absence of nanoplastic, were inversely proportional to biofilm formation.

No clear data were obtained with plate wells BSA blocked. Overall, our preliminary results indicate a clear influence of nanoplastics on the biofilm formation and on the bacterial growth. Nanoplastics probably act as valid substrate to "capture" *Bifidobacterium*.

Further studies by LC-mass spectrometry and NMR will be performed to investigate nanoplastic influence on Bifidobacterium metabolites production.

Characterization of micro- and nano-plastic interactions with biological systems through NMR methodologies

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The universal presence of micro- and nano-plastics and their unknown effects on the various biological systems are, to date, a significant concern.
Plastic debris can be fragmented into smaller pieces by many physical and chemical processes, generating its own micro- and nano-plastics. Recently, this debris was shown to affect biota and to be gradually spreading through the food chain, becoming dangerous to humans[1].
Therefore, the aims of this project are: from a microbiological point of view, to study the effect of micro- and nano-plastics on the activity of probiotic bacterial colonies, in particular *Bifidobacterium bifidum* colonies; from a chemical point of view, to study structural changes and interactions with biological macromolecules by focusing on one of the most important and well-known proteins, the human ubiquitin, using solution and solid-state NMR. In particular, in this latter case NMR data will be combined with HR-MAS, CD, DLS, DOSY, dynamics and molecular modeling data in an integrated approach. Finally, we will try to understand the effects of these polymers in biological fluids, through proteomics and metabolomics analysis, and cell-interaction studies using in-cell NMR methologies.

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Structural characterization of multi-domain proteins involved in neuronal diseases

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The focus of the PhD project is the structural and dynamics characterization of the human prion (HuPrp) and Phox2B proteins using NMR, to understand their role in neuronal diseases. During the first year, my research activities have been focused on the description of the folding mechanisms of $HuPrp^{23-231}$ (full-length) and $HuPrp^{90-231}$ under different pH conditions and in the presence/absence of Cu^{2+} by using an integrated NMR-based approach. The prion protein consists of a largely unfolded N-terminal portion (residues 23-124) and a folded C-terminal domain (residues 125-231), containing three ✔-helices and a short ☙-sheet. NMR studies have also demonstrated that the C-terminal domain structure is preserved even in the absence of the N-terminal domain ($HuPrp^{90-231}$). The misfolding of PrP^{C} into PrP^{Sc} may occur due to genetic mutations of the PrP gene enhancing the aggregation propensity of the protein or through infection by diseased PrP^{Sc} forms, which then act as a template for PrP^{C}-PrP^{Sc} autocatalytic conversion [(1)]. Nonetheless, most reported prion-pathies are the results of spontaneous conversion of PrP^{C} into PrPSc whose mechanism has been not yet elucidated, despite the fact that several in vitro and computational studies suggest PrP high conformational flexibility as a crucial factor in aggregation mechanism. The main goal of this study is to understand the structural and dynamics determinants controlling the formation of intermediate states involved in fibril assembly. The preliminary NMR studies indicate that the N-terminal disordered region transiently interacts with the C-terminal domain. Additionally, NMR and circular dichroism (CD) data demonstrate that the deletion of the N-terminal domain induces a variation of the folding pathway passing from a simple two-state process to a more complicated folding mechanism through the formation of a stable intermediate state at 334 K. Successively, to better understand the role of the intrinsically disordered domain in the modulation of the folding mechanisms of multi-domain proteins, I have focused my attention to a structurally analogous protein, Phox2B, which is a transcription factor playing an essential role in congenital central hypoventilation syndrome (CCHS)[(2-4)], caused by the presence of a polyalanine (polyAla) region that is the principal mediator of the protein aggregation. Here, in order to describe the role of the polyAla stretch in the protein aggregation involved in fibril assembly, the structural characterization of Phox2B containing the correct C-terminal (20 alanines) stretch by using NMR spectroscopy has been started. A virtually complete assignment of the backbone chemical shift has been obtained by using the standard triple resonance NMR experiments and binding experiments were carried out between the protein in the full length form and of the globular domain with double-stranded DNA sequences, to better understand the DNA-recognition mechanism with the protein.

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Preparation of devices from natural substances for controlled release

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In the last years, natural origin materials are finding more and more space as source for the design of new devices for the controlled release of active principles, both in biomedical field and in agriculture applications. This approach aims to reduce the impact of drugs and pesticides on human health, by combining the controlled release strategy with biocompatible and biodegradable carriers. To achieve this, carbohydrates are considered very advantageous polymeric material, thanks to their natural abundance. In addition, carbohydrates can be easily chemically modified, in order to obtain manifold different morphologies and structures. In this research activity, carbohydrates both of plant and fermentative origin, such as cellulose and hyaluronic acid, were evaluated for the preparation of controlled release systems with different structures (nano/microparticles, hydrogels, and water suspensions). The composition of the new materials was optimized through physical chemical and rheological analysis and subsequently their biological properties were evaluated. Finally, for future applications in biomedical and agricultural field, the produced devices were loaded with active principles, chosen among drugs and pesticides of natural origin.

Exciton and Charge Separation : Computational Models

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G-quadruplexes (G4) are four-stranded globular nucleic acid secondary structures formed in specific DNA G-rich sequences. Direct absorption of UV radiation can lead to oxidative damage, involving the generation of Guanine radical cations that can be metastable towards the loss of a proton, either that is bonded to N1 or N2 atom [1]. During my second year of Ph.D, I focused mainly on two complementary research lines. On the one hand, I have continued the study of the effects that the presence of an ionized G base has on the structure of the human telomeric sequence, Tel21(GGG(TTAGGG)3) using classical MD simulations. The study has been carried out in collaboration with Šponer and his co-workers who developed the parameters for G cation within the OL15 force field [2]. We show that the effect of G+ on the structural behavior of the Tel21 depends on its position in the sequence and that in some cases dramatic structural rearrangements are predicted.
On the same time, I contributed to the development of a new excitonic model, the Fragment Diabatisation based Excitonic Model (FrDex), for the study of strongly coupled multi-chromophore systems, using the calculation of the Electronic Circular Dichroism spectra of the same human telomeric sequence. This exciton model incorporates Charge transfer coupling and intra-molecular diabatic coupling as compared to Frenkel hamiltonian models [3]. Using this model, we could reproduce the ECD spectra very close to full TD-DFT and they are also in good agreement with experimental spectra, with a fairly small computational cost.

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Specialized metabolites as potential lead compounds for anticancer drug discovery

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Cancer is a serious public health problem worldwide with high incidence and mortality rates: 9.6 million people of 18.1 million new cases diagnosed have died in 2018[1]. Despite being one of the most frequently used by standard therapeutic approaches for cancer therapy, the prognosis and outcome of chemotherapy are still far from satisfactory due to the adverse side effects. The rising burden of cancer need the development of novel and alternative treatments[2].
Specialized metabolites (also termed "secondary"), produced in response to biotic and abiotic stress by plant, insect, and bacteria showing diversity and structural complexity, represent a rich source of lead compounds in their initial structural forms, as derivatives or as semi-synthetic molecules that could be applied in cancer chemotherapy[3,4].
In this project, NMR-based metabolomics is used to provide a snapshots of plant sample metabolomes: 1D NMR is classically used to gain an overview of the major compound families and 2D NMR for structural characterization and elucidation of compounds. Alone or in combination with other analytical technologies such as MS, NMR is useful to investigate new compounds or new sources of known anticancer compounds by plant extract or by plant pre-purified sample (using SPE or other purification methods)[5].

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New strategy based on polymeric biomaterials combined to stem cells to promote pulmonary tissue regeneration

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Lung diseases (LDs) are characterized by derangements of the alveolar walls and altered alveolar functions, causing an impaired respiratory function. The palliative effect of current therapies leads to an aberrant repair process of lung tissue. New hope for the treatment of LDs have emerged from Mesenchymal stem cells (MSCs) and their secretome, due to their ability to restore lung function, acting via paracrine mechanisms. However, the aberration of lung extracellular matrix (ECM), occurring in LDs, can preclude the normal tissue repair and the correct MSCs homing. Indeed, ECM provides a functional role and mechanical support to the pulmonary environment. This research activity aims to develop injectable polymeric biomaterials able to mimic the natural pulmonary ECM, combining with MSCs and their secretome, to support altered ECM and improving the delivering and the homing of the MSCs in the damaged lung, promoting the pulmonary tissue regeneration. To this purpose, injectable biomaterials as collagen solution, able to gel in situ at the physiological temperature, and solutions of hyaluronic acid (HA) have been employed. Cell viability, rheological characterization and the penoumocyte differentiation of MSCs have been assessed. Results demonstrated that MSCs, once exposed to the biomaterials contact, undergo a positive effect on pneumocytic differentiation.

Molecular characterization of the interaction of PREP1 and MEIS1 with PBX1

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The tumor –suppressor PREP1 (Pbx-regulating protein1) and the oncogene MEIS1 (Myeloid ecotropic insertion site1), are transcription factors belonging to the TALE (Three Amino acids Loop Extension) family, involved in the embryonic and cancer development [1-3]. PREP1 blocks the oncogenic function of MEIS1, by competing for PBX1 (Pre-B-cell leukemia transcription factor1)-binding in the cytoplasm, which regulates their nuclear translocation and directs the DNA binding to specific and only partially overlapping sequences [4-5].

Despite the crucial role of PREP1-PBX1 and MEIS1-PBX1 complexes, little is known about the molecular recognition mechanism between the proteins. Unveiling the PBX-interaction surface of PREP or MEIS would enable a better understanding of their molecular functions and lay the foundation for searching therapeutic molecules.

In this framework, cross-linking experiments between the two complexes coupled with mass spectrometry (MS) analysis were performed. Therefore, several mutant proteins were expressed and structurally characterized and their ability to interact with each other were assessed by ELISA and label free assays.

Results provide information on the relative importance of domains and residues involved in the mutual interactions and suggest protein area that may be targeted to specifically inhibit MEIS1 or PREP1 interaction to PBX1 [6].

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Secondary metabolites from Mediterranean plants for nutraceutical and pharmaceutical applications

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Secondary metabolites (SM) represent a library of bioactive compounds useful for humans like possible nutraceuticals and/or pharmaceuticals.
We focus our attention on *Cistanche phelypaea* (L.) and on four Sardinian plants *Myrtus communis*, *Scrophularia trifoliata*, *Helichrysum saxatile* and *Plagius flosculosus*. This study is heavily supported by 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 most promising *Cistanche*'s water extracts were evaluated for enzymatic inhibition related with the onset of acetylcholinesterase and butyrylcholinesterase, type 2 diabetes mellitus and skin hyperpigmentation. These extracts are rich source of iridoids and phenylethanoids (PhGs). The most promising PhGs (echinacoside and tubuloside A) were than tested for docking toward the selected enzymes used for the biological tests.
In Sardinian plants, feruloylquinic acids, acylphloroglucinols, iridoids and spiroketals are the main identified SM. MTT assay[1] was performed in order to evaluate the possible extract's anti-proliferative activity on liver cell lines. Subsequently, other tests also assessed their potential antimicrobial[2] and anti-HIV. From the main identified compounds spiroketals from *P. flosculosus* and acylphloroglucinols from *M. communis* were the most promising compounds.

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Different Impacts of MucR Binding to the babR and virB Promoters on Gene Ex pression in Brucella abortus 2308

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Brucellosis is one of the most widespread zoonoses worldwide that causes abortion in cattle, sheep and goats which constitutes a serious threat to the livestock industry and a source of human infection.
Brucella spp. are the bacteria responsible for brucellosis. MucR protein from B. abortus has been described as a transcriptional regulator of many virulence genes.
Deletion of the gene encoding for MucR produces attenuated Brucella strains, confirming the pivota l role of this protein in virulence.
MucR is a member of the Ros/MucR family comprising proteins that control the expression of genes necessary for the successful interaction of α-proteobacteria with their eukaryotic hosts.
Using electrophoretic mobility shift assay we show that MucR protein plays a direct role in regulating the expression of the Lux-like type regulator BabR by binding multiple target sites present in the babR promoter. Our in vitro experiments also demonstrate that MucR can bind the virB promoter, but with a lower affinity compared to babR target sites.
Moreover, MucR modality to bind AT-rich sequences containing T-A steps present in the promoters analyzed, supports the previous hypothesis that this protein is a histone-like protein involved in gene regulation through chromosome organization.

Targeting Nodal and Cripto-1 onco-fetal proteins using recombinant antibody fragments decorated Nanoparticles

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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 poorly present in normal adult cells. Re-expression of these proteins in the adults are associated with many tumors where they can control intracellular signaling and promote tumorigenesis [1]. The two proteins have been thereby indicated as potential diagnostic biomarkers and therapeutic targets for several types of cancer. We have already generated anti-Nodal and anti-Cripto-1 monoclonal antibodies with proven therapeutic efficacy [2][3][4]. Together these mAbs also may take advantage of working in combination acting as potential molecules grasped as single agents with dual or multi-targeting features that may act as double neutralizing molecules to block the whole Nodal/ Cripto-1/Alk4 axis. To obtain molecules with better PK/PD profile, we have designed and produced chimeric and humanized recombinant Fabs of anti-Nodal and anti-Cripto using E. coli as the host [5]. Furthermore, these recombinant Fab fragments have been opportunely engineered for site specific bio-conjugation via MTGase enzyme. We present the biochemical characterization of efficiently produced recombinant fragments in term of folding (Circular Dichroism), identity (ESI TOF mass spec) and binding analyses (Western Blot, ELISA, SPR, Bio-Layer Interferometry) and site-specific labelled Fabs. As proof of concept, these recombinant antibody fragments have been combined to generate bispecific Fab2 molecules by joining them through suitable synthetic linkers or co-tethered on 100 nm Human Serum Albumin nanoparticles, HSA-NP [6]. The recombinant antibody fragments alone, their bispecific variants and the Fab-decorated HSA-NPs are new valuable tools for both imaging and therapeutic purposes in Cripto-1/Nodal positive tumors.

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Zinc finger and the metal: xenobiotic ion as structural and folding cofactor in the prokaryotic zinc finger Ros87

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Metalloproteins must bind their metal cofactors with suitable affinity to carry out their biological function; even when the cofactor plays an exclusive structural role, it is essential to protein activity. For this reason, great attention has been devoted to the influence of xenobiotic metal ions on the structure, folding mechanism, and functions of metalloproteins.[1,2]
In the case of zinc finger domains, both eukaryotic and prokaryotic, the active fold is achieved by combining the structuring effect of the zinc cofactor and the formation of a hydrophobic core[3] that for a prokaryotic domain is an extensive hydrophobic core and contributes to the folding of a larger domain.
Here, we will report the results of studying the structural and functional effects of xenobiotic metal ion replacement in the coordination sphere of the prokaryotic zinc finger Ros. We have explored the binding of Co(II), Ni(II) and Cd(II) to Ros87, the DNA binding domain of the prokaryotic zinc finger Ros, by Uv-Vis, CD, DSC and NMR techniques. We discuss the difference of binding affinities for the different metals shown by Ros87 in relation to the differences in structure and folding mechanism and try to find a rule to the issue of zinc finger/xenobiotic metal ion functional tolerance.

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Biochemical and biophysical studies of the DNA helicases DDX11 and FANCJ involved in G-quadruplex nucleic acid metabolism

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Faithful transmission of genetic information is crucial for survival and is performed by the replisome. Cells are equipped with a set of proteins responsible for binding and destabilising DNA secondary structures that can be impediments for the accurate genome duplication. G-quadruplexes (G4) are one of these structures: they arise from guanine-rich sequences that fold into stable planar G-quartet stacks. These alternative DNA structures cause replication stress and genetic/epigenetic instability. FANCJ and DDX11 are DNA helicases implicated in cancer development and rare genetic syndromes. Both are able to dismantle G4 DNA structures *in vitro* with different specificity, but the physiological relevance of this activity is not known. They associate with the replisome through direct interaction with the replication factors AND-1 and Timeless, respectively. These proteins are all involved in processive replication of difficult-to-replicate templates, where stable secondary structures are present (such as G4, triplexes and hairpins). During my project, I plan to investigate the interplay of DDX11 and Timeless in resolving the above unconventional DNA structures using a combination of biochemical analyses with *in vitro* reconstituted systems and single-molecule biophysical studies using correlative optical tweezers – fluorescence microscopy techniques. Besides, I intend to further investigate the newly discovered FANCJ:AND-1 interaction and assess its relevance to various genome integrity maintenance pathways.

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 a wide group of neurodevelopmental disorders (NDDs) characterized by recurrent early onset infantile spasms (IS) and neurodevelopmental deficits, evolving in pharmacoresistant epilepsy in the adulthood.
One of monogenic causes of EEs are expansion mutations in a GCN codon repeat of the *Aristaless* related homeobox (*ARX*) gene, that lead to a polyalanine (polyA) tract elongation, and, subsequently, ARX protein function impairment. *ARX* is a X-linked gene codifying for a bifunctional transcription factor with a crucial role in the correct development and migration of cortical interneurons.
We report on *in vitro* studies performed in embryonic stem cells (ES) ablated in *Arx* (*Arx*-KO) showing how small botanically derived molecules impact on molecular and functional defects detected in GABAergic-oriented neuronal cells. Moreover, we show results on characterization of cortical microcircuitry in a polyA mouse model that recapitulates the epilepsy phenotype of *ARX* patients. By using *Arx*-polyA/ParvCRE mice and Adeno-Associated Virus (AVVs) tracing approaches, we evaluated intracortical connections *via* corpus callosum. Moreover, we performed immunofluorescence experiments to evaluate the composition of parvalbumin positive interneurons. Finally, we studied by whole-cell patch clamp recordings the intrinsic properties of cortical neurons in mutant and wild type brain slices. Preliminary data on the abnormalities of excitatory/inhibitory profiles have been obtained.

Structure and function of key macromolecules involved in severe humane pathogeneses

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Through structural biology, my PhD studies revolve around the modulation of the immune system is a strategy to tackle diverse human pathologies.

CD55 is a protein that regulates the complement system of the innate immune response. Its overexpression observed in several pathological conditions (including several cancers) is thought to be a mechanism to avoid the complement-mediated cell lysis. We developed and produced several molecules able to bind tightly to CD55 to inhibit its action. Using biophysical techniques, we have proved that the developed molecules bind CD55 with high affinity. Currently, we are measuring the binding affinities of these molecules to cancer cells. Although these molecules are very interesting from a therapeutic standpoint, they can be considered also an interesting diagnostic tool in pathologies associated with the overexpression of CD55.

Rv2299c is a protein from *M. tuberculosis* that was identified by our collaborator Prof Hwa-Jung Kim from the University of Daejeon. This protein displays promising antigen properties in immunization against tuberculosis, but its structure and function were hitherto unknown. We identified this protein as a chaperone with ATPase activity and devised a structural model for based on a homologue from *E. coli*. Through this model, we identified a putative mode of action of Rv2299c. Furthermore, we designed a fusion protein with another *M. tuberculosis protein* and observed heightened antigenic properties and decreased toxicity for this already interesting antigen.

Session 4:

Molecular Cell Biology

Exploring the roles of DDX11 and FANCJ DNA helicases in genome stability maintenance pathways

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DDX11 and FANCJ are both iron-sulfur (Fe-S) cluster-containing DNA helicases that belong to the super-family 2 (SF2). In humans, mutations in DDX11 and FANCJ are linked to rare genetic disorders, Warsaw Breakage Syndrome (WABS) and Fanconi Anemia (FA), respectively, that are characterized by genomic instability.
Both helicases are associated to the ongoing replication forks and are thought to be implicated in the resolution of alternative DNA structures (such as G-quadruplexes, G4) that arise at G-rich regions, like the ribosomal RNA genes. These latter are clustered at particular chromosomal sites, termed nucleolus organizer regions (NORs), in each of the five human acrocentric chromosomes.
The role of DDX11 and FANCJ DNA helicases in assisting smooth progression of the replication machinery at these difficult-to-replicate genomic loci is not well understood. Therefore, I plan to analyze the karyotype of DDX11- and FANCJ-KO mammalian cell lines to examine if any phenotypic anomaly (such as cohesion defects, gaps, breakages) can be associated with specific chromosomes. I will also use immunofluorescence-based techniques to visualize ultrafine anaphase bridge (UFB) formation at specific chromosomal regions. Rescue experiments will be carried out using the above KO-cell lines complemented with wild type DDX11/FANCJ and their mutant forms of interest.

Mitochondrial dynamics as a new therapeutic target for neurodegenerative diseases

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Mitochondrial impairment is considered a common feature in primary fetal fibroblasts with trisomy 21 compared to euploid ones. Thus, drugs improving mitochondrial function, like metformin, are proposed as a possible therapeutic intervention. Metformin may act both by promoting mitochondrial biogenesis and inducing the autophagy/mitophagy pathway. To assess the effect of metformin treatment on autophagy pathway, the protein levels of common autophagic markers were evaluated via western blot. Chloroquine treatment was introduced to bypass the high variability of basal autophagy noted in the euploid and trisomic samples. The chloroquine effect on autophagic markers along with mitochondrial morphological parameters was further evaluated on confocal image datasets. Following 3D pixel-based object segmentation in FIJI, intensity and morphometric measurements were extracted. Both volume and intensity values of LC3B+ punctae were increased after chloroquine treatment, regardless of karyotype, and p62+ punctae showed a similar trend in immunofluorescence. Mitochondria appeared swollen and fragmented after chloroquine treatment, while the combinatorial use of chloroquine and metformin did not provide significant differences among the samples with different karyotype. The chosen metformin dosage was not able to reverse the altered mitochondrial phenotype, keeping in mind the short (24h) treatment period. An increased metformin dosage might prove beneficial, requiring further exploration.

HUMAN INDUCED PLURIPOTENT STEM CELLS AS MODEL TO STUDY NEURODEGENERATIVE DISORDERS: A FOCUS ON SPINOCEREBELLAR ATAXIA TYPE 17

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The aim of my PhD project is to investigate, through a multidisciplinary integrated approach based on cellular models, molecular mechanisms and metabolic alterations underlying several neurological and neurodegenerative disorders, with a focus on Spinocerebellar ataxia type 17 (SCA17). In particular, we will make an observational and interventional in vitro study of fibroblasts and neurospheres, neural stem cells derived from the spontaneous differentiation of human induced pluripotent stem cells (hiPSCs) obtained through fibroblast reprogramming. The first part of the project is focused on the establishment of hiPSC lines. Fibroblasts were derived from the basal lamina of biopsies obtained from several patients and healthy controls. Then fibroblasts were reprogrammed to hiPSCs through a virus-free and feeder-free approach and, finally, the hiPSC clones were characterized in terms of gene expression, karyotype and STR analysis. The following step was the neuralization, a differentiation protocol able to produce neurospheres from hiPSCs. These cellular models will allow us to investigate the in vitro potential efficacy of therapeutic or protective factors towards the issues analyzed.

Molecular, biochemical and behavioural characterization of *D-aspartate oxidase* knock-in mouse model

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In mammalian central nervous system, free D-aspartate (D-Asp) occurs at high concentrations in the embryonic phase and decreases after birth. D-Asp is an endogenous agonist for NMDA and mGlu5 receptors. However, biological significance of D-Asp occurrence in mammalian brain is still unknown. Recently, it was also reported that D-Asp treatment stimulates the maturation of oligodendrocyte precursor cells and attenuates demyelination. In order to better investigate the role of D-Asp in myelination processes in the spinal cord, we generated a knock-in mouse model in which the enzyme responsible for D-Asp catabolism, D-aspartate oxidase (DDO), is overexpressed starting from the zygotic stage, to enable the removal of D-Asp in prenatal and postnatal life. As a proof of the successful achievement of our gene targeting strategy, Real-Time PCR experiments revealed increased *Ddo* mRNA levels in the spinal cord of *Ddo* knock-in mice during pre and postnatal ontogenesis. Accordingly, HPLC analysis showed a dramatic decrease of D-Asp content in the spinal cord of *Ddo* knock-in mice at all ages analyzed. Finally, we reported that *Ddo* knock-in mice did not show alterations in spontaneous exploration and coordination.

D-aspartic acid upregulates DAAM1 and PREP expressions during spermatogenesis

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D-Aspartic acid (D-Asp) is involved in mammalian spermatogenesis. In this project we studied the role of D-Asp in the cytoskeleton remodeling which occurs during germ cell differentiation. We performed in vivo experiments consisting of acute (i.p injection of 2 μmol/g b.w) and chronic (15 days drinking of 20mM solution) administrations of D-Asp to adult rats to investigate the expression of both Prolyl Endopeptidase (PREP) and Dishevelled-Associated Activator of Morphogenesis 1 (DAAM1) in rat testis. PREP is a protein belonging to the serine protease family, which has been identified as a binding partner of tubulin. DAAM1 is a formin-family protein involved in nucleation of unbranched actin filaments. The results demonstrated that D-Asp upregulated the expression of both PREP and DAAM in rat testis. PREP modulated spermatogenesis through activation of AMPA receptor and ERK and Akt pathways. Interestingly, the data also suggested a role of D-Asp in promoting DAAM1 shuttling to the nuclear compartment of proliferative cells. The proliferative action induced by D-Asp was confirmed by the increased levels of both PCNA, a protein expressed in the nucleus of cells in the S phase and p-H3, an important histone for chromatic condensation during mitosis and meiosis.

The short and the long RNAs at miR-99b/miR-let7e/miR-125a-Spaca6 locus: an open scenario in hepatocellular carcinoma

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MicroRNAs (miRNA), and more recently long non-coding RNAs (lncRNA), are emerging as key players in the pathogenesis of hepatocellular carcinoma (HCC). During my first and second PhD years, we investigated the roles of two oncosuppressive miRNAs (miR-125a and Let-7e) and a long non-coding RNA (SPACA6P-AS, SP-AS), all transcribed from the same genomic locus, with SP-AS in the opposite direction and thus carrying complementary sequences to the miRNAs.
In vitro experiments validated the binding of the miRNAs to SP-AS and their reciprocal expression inhibition. Importantly, over-expression of SP-AS reduced the silencing of miR-215a and let-7e toward their key oncogenic targets related to the control of cell proliferation in the HCC context, i.e. Lin28b, MMP11, SIRT7, Zbtb7a, Cyclin D1, CDC25B, HMGA2, that resulted significantly upregulated. Consistently, SP-AS over-expression counteracted the antiproliferative effects of both miRNAs. Exploitation of expression data contained in The Cancer Genome Atlas (TCGA) relative to 374 HCC samples in comparison to 50 normal liver tissues showed an upregulation of SP-AS and a reverse expression of miR-125a, not observed for let-7e; consistently, miR-125a oncogenic targets were upregulated. The data depict a novel competing endogenous RNA (ceRNA) network, ceRNET, whereby miR-125a can regulate the expression of SP-AS, which in turn regulates the miRNA by preventing its inhibitory binding to the target transcripts. Finally, we also investigated the regulatory loop occurring between miR-125a and its oncogene target Zbtb7a.

PARP12 as a Novel Target in Cancer Resistance to Chemotherapy

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PARP12 is a mono-ADP-ribosyltransferase of the PARP family, with regulatory roles in membrane trafficking and cellular stress response [1,2]. Along with these functions, PARP12 has been identified as a key factor in breast-cancer resistance to chemotherapy by contributing to tumour survival and re-growth [3]. To evaluate this further, we studied the PARP12 depletion effects in breast-cancer cell lines in comparison to the non-tumorigenic counterpart. Our lab results showed that PARP12 depletion by siRNAs promotes apoptosis selectively in tumoral cells, as detected by FACS analysis and PARP1 cleavage. We also found that Akt, a major regulator of cell survival [4], is ADP-ribosylated by PARP12. In order to elucidate the molecular events that induce apoptosis in MCF7 cells upon PARP12 depletion, I am validating putative Akt ADP-ribosylation defective mutants through Af1521 *macro* domain pull-down assay, a method to follow intracellular ADP-ribosylation, and functional assays [5]. Further we started to generate PARP12 knockout cell lines through CRISPR/Cas9 technology to validate PARP12 sensitivity and tumour induction in *in vivo* models. These studies will improve our knowledge in understanding the role of PARP12 in breast cancer resistance to chemotherapy and will be instrumental in developing PARP12 inhibitors as novel candidates for breast cancer treatment.

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Effect of 3,5-diiodo-L-thyronine (T2) administration on visceral white adipose tissue inflammatory response in high-fat diet-induced overweight rats

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T2, an endogenous metabolite of thyroid hormones exhibits beneficial metabolic effects. Its actions exerted in counteracting fat accumulation in rats on a high fat diet (HFD) are particularly relevant. Visceral white adipose tissue accumulation is associated with the inflammatory response that plays an important role in the development of many diseases related to obesity. In this contest, many cytokines are released by inflammatory cells infiltrating adipose tissue and all of these molecules may act leading to local and generalized inflammation. The present study aimed to demonstrate whether T2 is able to improve the inflammatory response of adipocytes in overweight rats. Three groups of rats were used: i) receiving a standard diet for 14 weeks; ii) receiving a HFD for 14 weeks, and iii) receiving a HFD for 14 weeks with a daily injection of T2 for the last 4 weeks. The results showed that T2 administration increased tissue anti-inflammatory cytokine expression, decreased inflammatory cytokine expression and reduced hypoxia. Furthermore, in T2-treated animals, there was a reduction in HFD-induced angiogenesis correlated to modulation of miR126 expression, a post-transcriptional regulator involved in controlling genes of the angiogenic pathway. In conclusion, this study shows that the effect of T2 on adipose tissue inflammation represents another action by which this iodothyronine counteracts overweight-associated comorbidities.

Pharmacological targeting of the protein CtBP1/BARS in cancer and in viral infection

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CtBP1/BARS, a C-terminal-binding protein (CtBP) family member, is bifunctional: in the nucleus represses transcription of genes with proapototic, tumor-suppressor and other cancer-relevant functions, while in the cytoplasm controls membrane fission in membrane transport (endo- and exocytosis) and in mitotic Golgi partitioning. Structurally, CtBP1/BARS belongs to the 2-hydroxyacid dehydrogenase family and possesses a Rossmann fold, which controls its conformation and cellular functions, depending on the bound ligand (NADH, acyl-CoA or small molecules/drugs). Both the CtBP1/BARS activities are cancer-related and can be targeted by small molecules using its Rossmann fold as pharmacological target.
We have previously identified 150 molecules by Drug Repurposing approach from a library of approved, "safe-in-man" drugs. I have started a functional screening identifying four drugs: Erdosteine, Lysofilline, Emitricitabine and Olopatadine, as the most active CtBP1/BARS inhibitors.
We have also identified CtBP1/BARS as a key controller of macropinocytosis, the endocytic pathway used by SARS-CoV-2 (like Ebola, EV1 and AdV3) for cell infection/internalization.
Here, we aim to identify at least one drug with sufficient potency and selectivity: i) in cancer models (administered individually or in combination with chemotherapeutics) to became a candidate for future clinical testing; and ii) in SARS-CoV-2 infection/internalization tests as candidate for COVID-19 treatment.

Unravelling new pathways and innovative perspectives for treating Duchenne Muscular Dystrophy: focus on the endocannabinoid system and its interplay with the gut microbiota

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Skeletal muscle dystrophies comprise a well-known group of hereditary disorders associated with a devastating deterioration of muscle tissues. Among these disorders, Duchenne's muscular dystrophy (DMD) is the most frequent form affecting about 1 in 3500 live male births. The disease is caused by mutations in the X-linked gene encoding for the structural protein dystrophin. Recent studies identified autophagy as an important homeostatic mechanism deranged in dystrophic muscles and indicate that novel therapeutic approaches aimed at reactivating autophagy are a valuable strategy to reduce muscle damage in DMD. The Endocannabinoid System (ECS) refers to a large group of molecules that in our body control the activity of the two major cannabinoid lipid-mediators named Anandamide (AEA) and 2-Arachidonoylglycerol (2-AG). Despite growing evidence pointing to the promising use of cannabinoids as complementary and/or alternative medicines, to date, there is lack of knowledge about the potential use of these molecules in skeletal muscle disorders or other human diseases associated with defective autophagy. Only recently our research group demonstrated that: (i) 2-AG promotes inhibits the maturation of skeletal muscle progenitors; (ii) there is an increased activity of the ECS in both murine and human skeletal muscles affected by muscular dystrophy; (iv) the pharmacological inhibition of the endocannabinoid CB1 receptor, significantly delays locomotor impairment in dystrophic mice by reducing inflammation and promoting autophagy. In addition, there is emerging evidence demonstrating that the functional interactions between the ECS and gut microbiota are crucial for host physiology, homeostasis, and sustained health. In this study, we demonstrate that in dystrophic mice, respect to healthy controls, there is a profound alteration of the gut microbiota composition. Along with these changes, in the plasma and skeletal muscles of dystrophic mice we found altered levels of key microbial metabolites including short-chain fatty acids (SCFAs) as well as of AEA and 2-AG. In murine C2C12 myoblasts, using pharmacological and gene silencing strategies, we demonstrated that the stimulation with the SCFAs or synthetic endocannabinoids reduces the expression of autophagy-related genes. In summary, our results indicate that in dystrophic mice there is an altered gut microbiota and endocannabinoid system tone importantly regulating the progression of skeletal muscle damage, inflammation and autophagy.

Fasting and exercise affect T4 and BDNF signaling pathways

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Nutritional interventions involving exercise are recognized as powerful medicine, and physicians increasingly encourage patients to exercise through medical prescription based on the strong epidemiological link between sedentary lifestyle and various diseases. The beneficial effects of exercise and nutritional interventions not only regard peripheral tissues such as muscle and liver, but are also present at the central level. Thyroid hormone is an important stimulus for the response to exercise, and local levels of T3 can be modulated by the presence of monocarboxylate transporters Mct9 and Mct10. Mct1, in addition, is responsible for the uptake of ketone bodies as fuel. An important protein involved in the peripheral metabolic response to fasting and exercise, which is known to respond to altered ketone levels, is brain derived neurotrophic factor (BDNF). This protein also plays an important central role in cognition. To study in detail how a period of food deprivation influenced the outcome of a protocol of repeated mild exercise regarding the above factors, rats have been submitted to 5 bouts of exercise on a treadmill (speed, 15 m/min, 30 min each). Serum profiles of ketones and free T4 have been measured and transcriptional levels of the various Mct transporters have been investigated, both in muscle, liver and in brain. Results obtained in skeletal muscle , as well as results on the expression of BDNF and modulation of downstream signaling pathways in the prefrontal cortex will be presented.

Leprel-1 is involved in angiogenesis process

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Leprel-1, also known as prolyl 3-hydroxylase 2 (P3H2), is an enzyme that catalyzes the post-translational formation of 3-Hydroxyproline on collagens, acting mainly on collagen type IV. Its

activity has never been associated to angiogenesis, to date. We found that Leprel-1 expression is modulated by VEGF-A/VEGFR-2 signaling pathway in human umbilical vein endothelial cells (HUVECs). Furthermore, gain and loss of function experiments allowed as to demonstrate that Leprel-1 modulates HUVECs migration.
In order to investigate which downstream mediators of VEGF-A/VEGFR-2 signaling cascade is involved in the modulation of Leprel-1 expression, we used specific inhibitors of main signaling mediators and we found that Leprel-1 transcription is modulated through VEGFR-2/p38 MAPK signaling cascade. Furthermore, we shown that Leprel-1 is directly involved in *in vitro* angiogenesis performing capillary sprouting assay with HUVECs endothelial spheroids, in which was induced gain or loss of function of Leprel-1. Moreover, we performed immunofluorescence studies demonstrating that Leprel-1 overexpression induced collagen IV condensation.
The data obtained until know confirm a direct involvement of Leprel-1 in angiogenic properties of human endothelial cells. To evaluate its role in *in vivo* angiogenesis, two different models will be used. First, we will study the effect of Leprel-1 modulation in the model of laser-induced choroidal neovascularization (CNV). Second, since Leprel-1 has also been described as tumor suppressor gene and we have already confirmed its downmodulation in several colorectal cancer cell lines, we have generated HCT116 stable cells line to perform xenograft tumor growth experiments.

Identification of environmental and genetic cues that modulate neuron degeneration in *C.elegans*

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Several neurodegenerative diseases, including Parkinson (PD), have been linked to genetic and environmental factors. PD is characterized by the loss of striatal dopaminergic neurons, and affects

more often men than women. However how environmental conditions, gender and genetic variations can modulate pathological manifestations is unknown. To understand these interactions, I focused my attention on two PD models in *C. elegans:* the first overexpresses α-synuclein, a protein that can misfold and polymerize to form toxic fibrils coalescing into pathologic inclusions; the second overexpresses the G2019S mutated form of LRRK2 kinase, one of the genes that is most frequently involved in PD. In the first I tested the protective effects of *Vigna unguiculata* bean extracts against neurodegeneration (under review in *Aging*). On both models I tested how gender can modulate the neurodegeneration and found that dafachronic acid is involved in the $LRRK2^{G2019S}$ mediated sex-specific neurodegeneration. These results allowed to identify the molecular mechanisms causing the sex-specific differences in PD and to suggest new therapies for differential treatment of patients.
I also studied how space radiations can modulate the nervous system in different animal models, including *C.elegans*, which lead us to write a review on this argument (under review in *Frontiers in Physics*).

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

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PhD Cycle: 33° cycle

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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 endo-lysosomes and plasma membrane (PM). Our lab aims to understand the cargo fluxes across these compartments, through the concept of 'CONTROL THEORY', where in each

subcellular secretory station (ER or the trans Golgi network) is treated simultaneously as an individualistic station and also as a part of the system with preceding or succeeding station. Any perturbations in the functioning of these stations is recorded by a sensor (cargo sensing protein), which initiates a readjustment mechanism through the controller mechanism (a signalling or transcriptional circuit that calculates the appropriate response to the perturbation) and helps restore homeostasis via effectors (kinase substrates and proteins expressed as a consequence of transcription).

We have previously identified a control system called AREX (**A**uto **R**egulation at **ER** e**X**it sites) that senses folded cargo loads at the ER exit sites (ERES) 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) 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) and PM localised lipid anchored GPI GFP is not under control of the canonical AREX cascade. We have found that the above mentioned AREX independent cargoes utilize a signalling cascade entirely different from that of VSVG. Moreover, we suggest that different cargo classes exiting the ER, use particular exit sites and regulate distinct cellular process downstream of the ER, which help in the further post translation modifications and/or transport events which are exclusive for the cargo subset type.

Physical exercise and fasting as modulators of potential biomarkers characterizing diabetes

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Diabetes is a global disease with a high mortality risk. Lifestyle is a crucial element, especially exercise has been shown to be an activator of metabolic pathways that contrast insulin resistance.

Although there is much experimental evidence supporting the beneficial metabolic effects of calorie restriction or fasting in combination with exercise, it is not known whether and how the combination of these interventions leads to improvements in the pathological picture of diabetes. This doctoral project is part of this context. The project will include a part in the company Tecno Bios where a study of the lipidomic and metabolomic framework of serum and tissues such as liver, muscle, adipose tissue and intestine will be carried out. Applying an integrated metabolome/transcriptome analysis system will identify the different metabolic pathways related to lipid metabolism, including gluconeogenesis, mitochondrial dysfunction, oxidative stress and amino acid synthesis; Free bioinformatics platforms such as the Plugin Metscape for Cytoscape will be used. During the period abroad, studies of muscular performance in structural and functional terms will be carried out through optical and electronic microscopy analysis for the characterization of sarcomere in response to processes of autophagy and fiber type associated with metabolic changes induced by various treatments.

miRNA role in the genotype-phenotype relationship of X chromosome aneuploidy syndromes

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My PhD project aims at investigating microRNA role in X-chromosome aneuploidy syndromes, i.e Turner and Klinefelter syndromes, wherein no obvious karyotype-clinical traits relationship exists. Genomic distribution analysis revealed the highest density of miRNA sequences on the X

chromosome; this evolutionary conserved mammalian feature equips females with a larger miRNA machinery than males. However, miRNAs contribution to different X-related conditions, properties or functions is still poorly explored. For this purpose, the project is intended to accomplish a very large platform of miRNoma data for multiple comparisons aimed at identifying miRNAs involved in clinical traits of the syndromes, followed by functional analyses of selected miRNAs. First, a fine map of miRNA sequences on the X chromosome and possible modulators of their expression has been established, and bioinformatics functional analyses of the whole X-linked miRNA targetome were performed. Then, blood samples, adipose and muscle biopsies from Turner syndrome and Klinefelter syndrome adult and pediatric patients and healthy volunteers have been collected. The workflow involves miRNAseq analyses, identification of miRNAs differentially expressed, experimental target validation by cell cultures experiments; back to patient cohorts to analyze possible reverse expression correlation of miRNA-targets, further validating the functional role of those regulatory networks. This study could correlate miRNA-target networks to specific clinical traits to make sufficiently meaningful karyotype-phenotype associations, with important implications in terms of diagnostics and predictive medicine and development of innovative therapeutic approaches.

Characterization of adiponectin in cerebrovascular fluid from patients affected by Multiple Sclerosis

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In my PhD project, adiponectin expression and its oligomeric distribution were investigated in cerebrospinal fluid (CSF) of patients affected by Multiple Sclerosis (MS), an autoimmune

demyelinating disease of the central nervous system. Adipose tissue is strongly associated with development and progression of immune disorders through adipokines secretion. Adiponectin has beneficial properties in inflammation and immune processes; this adipokine circulates as oligomers of different molecular weight: HMW, MMW and LMW.
In this study, we analyzed total CSF adiponectin and its oligomeric profile by ELISA test, Western Blotting and FPLC chromatography in MS patients compared to age-and sex-matched controls. Our preliminary results show that total CSF adiponectin is statistically increased in MS patients compared to controls and positively correlates with progression and severity of the disease. We also found that adiponectin oligomerization state is altered in MS, with an increase of HMW and MMW oligomers. In conclusion, our study detected a modulation of adiponectin in CSF of MS patients and confirmed its role into MS pathogenesis suggesting that this adipokine could participate in the neurodegenerative process of MS disease regulating the inflammatory process typical of MS. Further studies are needed to clarify the molecular mechanisms underlying its activity.

Session 5:

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. 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. A recent form called 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 (A mutation abolishes ubiquitin binding capacity of NEMO). WE also noticed that 2 population of cells are present: a population in which RIPK1 interacts with RIPK3 (through a conserved region known as RHIM domain) and the second one in which NEMO interacts with RIPK3. We further characterized this interaction by inducing RIPK3-RHIM deletion (RIPK3-RHIMdel) and immunoprecipitated against NEMO to find that NEMO still binds to RIPK3. A possible intermediate of this interaction is IKKs so we immunoprecipitated in presence of IKKalpha & beta which had no interaction with RIPK3. Collectively, NEMO interact with RIPK3 possibly in a complex in which RIPK1, IKK complex seem to have no role. What functions are served by this interaction is still yet to be discovered. 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.
We also have done a systematic review (a preclinical animal model review) about the possible roles of necroptotic inhibitors (especially RIPK1 inhibitors) in treatment of neurodegenerative diseases like Alzheimer's disease, Multiple sclerosis, Amyotrophic lateral sclerosis and Stroke. The analysis of data extracted from this review suggests that inhibition of Necroptotic pathway could serve as an effective option for the treatment of CNS-related human diseases. We hypothesized that this strategy could also be used for the treatment of CNS manifestations seen in patients with a rare genetic and microvascular diseases that involve a CNS component (30 %) like Incontinentia pigmenti (IP).

Generation and characterization of murine models in imprinting disorders

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The H19/IGF2 IG-Differentially Methylated Region (IC1) regulates the reciprocal imprinting of *H19* and *Igf2*, and its abnormal methylation and genetic variants are frequently associated with the Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS). We have previously generated two transgenic mouse lines by replacing the endogenous mouse IC1 (mIC1) with the orthologous human sequence in either wildtype (hIC1wt) form or carrying a 2.2 kb deletion (hIC1Δ2.2) found in

BWS familial cases. The mice carrying the hIC1Δ2.2 allele showed tissue-specific and mosaic imprinting alterations resulting in pre/post-natal overgrowth (BWS-like phenotype) or undergrowth (SRS-like phenotype) on maternal and paternal transmission, respectively. Histological analysis indicated that the altered size of several organs may be due to a different amount of mucopolysaccharides in extracellular matrix, larger upon maternal transmission of the hIC1Δ2.2 allele, smaller upon paternal transmission.
RNA seq of kidneys revealed a higher expression level of genes involved in the inflammatory response pathway in 15% of mice carrying the hIC1Δ2.2 allele on the paternal chromosome, compared to their wild-type littermates. This increased expression, observed also in other tissues, correlated with higher H19 expression and lower H19 promoter methylation.

Also, we generated and characterized a third knock-in line carrying a 1.8 kb deletion of IC1, also found in familial BWS cases. Intriguingly, the hIC1Δ1.8 allele behaved more similarly to hIC1wt than to hIC1Δ2.2, on either maternal and paternal transmission. As hIC1wt, hIC1Δ1.8 properly regulates *H19* and *Igf2* imprinting and is associated with normal growth phenotype on maternal transmission. Also, complete lack of methylation and non-viable phenotype characterize its paternal transmission. Differently from hIC1wt, however, aberrant methylation of paternal hIC1Δ1.8 is unstable with stochastic events observed in one third of the mice.

The role of miR-181 in Parkinson Disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disease characterized by the progressive loss of dopaminergic neurons of substantia nigra (SN) pars compacta. Mitochondrial dysfunction has a prominent role in neurodegenerative events, especially in PD. MicroRNAs (miRNAs) are fine regulators of gene expression with a promising therapeutic role for their ability to concomitantly regulate different pathways. Over the past decade, dysregulation of miRNAs expression in PD has been reported. miR-181a and miR-181b (miR-181a/b) are highly expressed in the SN and striatum and enriched in the brains of PD patients. In the past, our group showed that

miR-181a/b regulate key genes involved in mitochondrial biogenesis and function (i.e. NRF1, PPARGC1A) and we now further demonstrate that miR181a targets TFAM which is regulated by NRF1. Interestingly, we find that miR181a/b inactivation has a protective effect in a 6-OHDA-induced mouse models of PD. Finally, we are now evaluating if miR181a/b could be considered as PD biomarkers. We are thus performing RT-qPCR to estimate miRNAs levels in peripheral blood of PD patients. In conclusion our preliminary results suggest that miR181a/b may represent both reliable and easy to measure biomarkers, and effective therapeutic targets in PD.

H3K4me3 profiles in subcutaneous pre-adipocytes of individuals with a family history of type 2 diabetes

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Background and aims: The risk of developing T2D is up to 10 times higher in individuals with family history (FDRs). FDRs have dysfunctional adipose tissue characterized by fat cell hypertrophy.

Epigenetic modifications may contribute to these abnormalities; therefore, we explored the role of H3K4me3 in contributing to T2D risk.
Materials and methods: Using a Genome-wide ChIP-Seq approach, we mapped H3K4me3 on the entire genome of preadipocytes isolated from 9 FDRs and 11 CTRLs. The detected epigenetic changes were validated using ChIP-qPCR experiments. Mitochondrial DNA (mtDNA) content and mRNA expression were assessed by qPCR.
Results: The differently H3K4me3 enriched regions, highlighted by bioinformatics analysis, were annotated and subjected to an enrichment analysis, which revealed the involvement of genes responsible for mitochondrial function and biogenesis (e.g. TFAM). After bioinformatical data validation, mechanistic studies showed a mtDNA content reduction in preadipocytes, a mitochondrial biogenesis block following adipocyte differentiation induction, and a reduction in the expression of genes controlling these mechanisms in FDRs. H3K4me3 levels on TFAM promoter in preadipocytes are related to glycemic control genes expression in adipocytes. These data indicate that alterations in histone modifications can cause mitochondrial dysfunction in the preadipocytes and contribute to an increased risk of T2D in FDRs.

DNA methylation defects at multiple imprinted loci in Beckwith-Wiedemann Syndrome and/or Wilms Tumor

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The Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome, characterized by variable clinical features and a high predisposition to nephroblastoma, also known as Wilms tumor (WT). BWS is caused by dysregulation of the imprinted gene cluster at chromosome 11p15.5. The cluster

is organized 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 (namely, ICR1 and ICR2), characterized by differential DNA methylation between the two alleles. Abnormal DNA methylation at these ICRs represents the most frequent molecular defect found in BWS and in WTs. A subgroup of BWS patients shows DNA methylation defects at multiple imprinted loci (MMDs) localized in chromosome regions other than 11p15.5. Which is the cause and the mechanism responsible of these epimutations in BWS is not completely clear. Additionally, whether the DNA methylation defects affect only 11p15.5 ICRs or also other imprinted loci also in WTs is still unknown.

Therefore, in order to investigate whether WTs can be affected by MMDs, as well as, to identify the cause underlying MMDs in WTs or BWS, we performed methylation analysis by pyrosequencing or MS-MLPA on DNA extracted from blood leukocyte of 44 BWS patients and from tumor tissue of 48 WTs. The results showed that 43% of BWS and 35% of WTs show MMDs. Interestingly, WT patients with MMDs show a more aggressive phenotype compared with other WTs. Next, we performed genetic mutation analysis by exome-sequencing or CGH-arrays to explore the possible cause of MMDs in both diseases. So far, we found that a small subset of BWS shows genetic variants in PADI6 and NLRP5 genes, components of the subcortical maternal complex. In contrast, CGH-arrays results suggest that the methylation defects of a subgroup of WTs are mainly caused by chromosome copy number alterations (deletions or duplications).

Genetic determinants of endogenous antioxidants variability in a population-based study

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Oxidative stress is the result of a mismatch between the excessive formation of reactive oxygen or nitrogen species and limited antioxidant defenses[1].
For our search serum endogenous antioxidant levels (including bilirubin, uric acid, thiols) have been assessed using commercially available test from Diacron International (anti-Roms Test) in a cohort consisting of 1455 individuals from three isolated villages.
A Genome-Wide Association Study was carried out to analyze the relationship between genetic polymorphisms in our sample and blood levels of endogenous antioxidants (anti-Roms serum measurements). In accordance with the capacity of the test to detect uric acid and bilirubin, we identified well-known associations for both these traits. To detect novel loci, anti-Roms levels were adjusted for those traits, identifying a statistically significant signal (rs2044982 in MS4A, P = 2.62E-08) in a gender stratified analysis.
Several studies have suggested that MS4A gene cluster plays an important role in the progression of Alzheimer's disease (AD). Recently, a variant at the MS4A locus was found to create an antioxidant response element linking MS4A6A expression to the response to stress[2].
These data encourage to validate the identified association in an independent cohort and to investigate the implication of MS4A locus in the oxidative stress and AD.

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Study of the molecular interplay between MeCP2 and AUTS2 in the glycosphingolipid metabolism and its involvement in Rett syndrome pathogenesis.

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MeCP2 is an epigenetic modulator of transcription highly expressed in neurons and mutated in Rett syndrome (RTT), a neurodevelopmental disorder with autistic features, and unknown pathogenetic mechanisms. We highlighted altered glycosphingolipid levels in *Mecp2*-null mouse brain. Moreover, MeCP2 modulates the expression of glycosphingolipid-related enzymes (GREs) and of AUTS2, a key regulator of glycosphingolipid reprogramming in neurons. MeCP2 defects cause AUTS2 up-regulation, with possible effects on RTT phenotypes.
We aim to dissect the role of MeCP2/AUTS2 crosstalk in glycosphingolipid metabolism, and to understand the effects of AUTS2 dosage in RTT pathogenesis, by generating new cellular and animal models.
We generated a *MECP2*-null SH-SY5Y human neuroblastoma cell line (*MECP2*$^{-/-}$) by CRISPR-CAS9 and the siRNA-mediated knockdown (KD) of AUTS2 in *MECP2*$^{-/-}$ cells is in progress. Moreover, we set up a protocol to further differentiate SH-SY5Y cells. We will analyze the content of glycosphingolipids and the expression of GREs in *MECP2*$^{-/-}$;AUTS2-KD cells, in comparison with *MECP2*$^{-/-}$ and control cells. These experiments will highlight the role of MeCP2/AUTS2 interplay in glycosphingolipid metabolism in human neuronal context, and the effects of AUTS2 dosage on this process. Moreover, we will generate a new mouse model *Mecp2*$^{-/y}$;*Auts2*$^{+/-}$ to understand the role of AUTS2 dosage in RTT pathogenesis.

A pipeline for prioritization of putatively damaging genetic variants

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I developed the GREP pipeline to prioritize putatively damaging genetic variants. GREP takes as input genomic variants information from cases and controls (including the per-individual allelic counts) in form of a vcf file and outputs a table of variants prioritized according to user-defined parameters. GREP uses functional annotations of genomic variants, information from publicly available sequence data of presumably healthy individuals, and, if available, knowledge of genes involved on the trait under study.
Based on the functional annotations in coding regions, the variants selected by GREP should match two criteria: posses an overall impact on the gene product classified as moderate or high by Ensembl and be putatively damaging in genes intolerant to loss of function. Information from the general healthy population is used to filter variants based on their allele frequencies and to test the chance of random occurrence of genes through resampling. Finally, it is possible to incorporate one or more user-defined lists of genes relevant to the trait under study and take this into account when selecting.
We applied the GREP pipeline to ten whole-genome sequences of miscarried embryo and nineteen exome sequences of mothers experiencing pre-implantation development arrest.

Identification of microRNAs involved in retinal cells degeneration and evaluation of their potential impact in the treatment of inherited retinal disorders

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Inherited retinal diseases (IRDs) are a clinically and genetically heterogeneous group of disorders, characterized by progressive photoreceptor degeneration and incurable loss of vision. MicroRNAs (miRNAs), a class of endogenously expressed non-coding RNAs with post-transcriptional regulatory properties, are known to play a major role in retinal function, both in physiological and pathological conditions. Since miRNAs are capable of simultaneously modulating multiple molecular pathways, they represent promising tools to therapeutically tackle disorders with high genetical heterogeneity such as IRDs. In the present work, a high-throughput screening approach was employed to study miRNAs' impact on a photoreceptor cell line undergoing light-induced degeneration. For this approach, more than 1200 miRNAs were transfected and assayed for their putative protective action in light-stressed 661W cone photoreceptor cells. Hereafter, miRNAs showing significant protective or destructive effects, will be individually tested with additional methods and the molecular mechanism underlying their role will be analyzed in *in vitro* and *in vivo* models. The identification of miRNAs exerting a relevant effect in this cellular system, could shed further light about the process of photoreceptor degeneration that remains unclear and, furthermore, lead to the development of novel therapeutic approaches for IRDs.

Therapeutic approach with Ataluren in Duchenne symptomatic carriers with nonsense mutations in dystrophin gene

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Timely and accurate molecular diagnosis in Dystrophinopathies is a crucial aspect of care. Diagnosis may be obtained in about 95% by testing peripheral blood DNA. In the remaining cases, a muscle biopsy should be taken to search for elusive RNA variants, such as those caused by deep intronic mutations. This mutations, unrecognized by DNA studies because of their unpredictable effects, cause aberrant dystrophin splicing and retention of pseudoexons in the final transcript.
In the most common case of deletion, a therapeutic approach based on exon skipping has been so far tested to restore the reading frame of a defective transcript. In the case of pseudoexon retention the same approach could be potentially more effective restoring a full transcript.
Our purpose is to recruit undiagnosed patients after DNA testing and provide them a genetic diagnosis through transcript analysis. We aim to investigate the splicing effect of deep intronic mutations, potential therapies by designing AONs and evaluating splicing modifier compounds.
Index cases of three families in our cohort underwent muscle biopsy. RNA was extracted and the junctions of aberrant spliced products allowed us to recognize the intronic causative variants. Their phenotypical characterization offers important hints for genetic counselling.

Hematopoietic differentiation of iPSCs derived from patient with the Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome into HPCs expressing CD45

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The ICF syndrome is a rare disorder causing immunodeficiency. Majority of patients carry mutations in DNA-methyltransferase-3B gene. Our aim is to elucidate the molecular basis of the immunological defects, investigating the morphological and molecular events occurring in induced pluripotent stem cells (iPSCs) during the induction of differentiation towards hematopoietic progenitor cells (HPCs). Specifically, we compared the results obtained from the differentiation of IPSC derived from ICF patients and healthy donors. Our protocol allows an efficient *in vitro* generation of HPCs in 12 days. Since day 10, we observed a higher percentage of apoptotic cells in ICF compared to wild-type cells, which was confirmed by FACS analysis at day 12. To characterize the HPCs generated from ICF-iPSCs, cells were analyzed by flow-cytometry for expression of the hematopoietic cell surface marker CD45. The percentage of CD45+ cells resulted significantly reduced in ICF compared to wild-type cells, indicating that the differentiation potential of disease iPSc is compromised. ICF and wild-type bulk and sorted populations were collected to perform expression studies at single gene and genome-wide level in order to enlighten transcriptional defects. In parallel, we are setting differentiation experiments including isogenic ICF-IPSc clones in which pathogenic mutations were corrected through CRISPR-Cas9 editing.

Linked whole genome sequencing as further step to study unsolved NMD cases

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NGS has changed our approach to neuromuscular disorders. Nowadays, one of the first step of the diagnostic process is to search for small mutations using panels of genes, WES or WGS. Even after WES/WGS, a percentage of cases remain negative, because of potential structural variants that require further testing; therefore, a new strategy is required. The recently developed linked-read sequencing technology, from 10X Genomics, combines a new barcoding strategy with Illumina NGS. In order to resolve NMD patients with WES-negative, we decided to use this strategy to detect variants such as small nucleotide variants (SNVs), indels, and large-scale structural rearrangements localized in complex regions of the exome. We previously adopted this new strategy to sequence a WES-enriched target, but the preparation step resulted not suitable for large scale-protocols, thus we replaced it with WGS. To this aim, a BMD carrier with a complex rearrangement of *DMD* gene was examined. In her family, a deletion of exons 16-29 in DMD gene was responsible for the disease. Unexpectedly, she showed a non-contiguous duplication of exons 1-15 and exons 30-34, clearly involving the flanking regions of the firstly identified family-deletion. Using linked-read sequencing technology by 10X Genomics, we found a duplication of 1-34 exons of the WT allele that restored the normal dosage of exons 16-29. These preliminary data suggest that this approach could be crucial to solve the undiagnosed NMD patients.