Università
degli Studi
della Campania
Luigi Vanvitelli

PhD Days 2019
PhD Programme in Molecular Life Science

Aula seminari Igb, CNR

Cancer biology, Immunology, Microbiology, Drug design

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

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

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

Giacomo Della Camera
Study of inflammatory response and complement activation following the use of engineered nanoparticles (ENPs).
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Maria Rita Milone
Repurposing of valproic acid and simvastatin as anticancer agents in pancreatic cancer: a new therapeutic approach in combination with chemotherapy.
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Fibril formation assay to test new halogenated compounds as transthyretin misfolding inhibitors
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Magdalena Kostrzewa
The Endocannabinoid System as a target for the treatment of bone disorders
Tutor: Dr. Fernando Gianfrancesco/ Dr. Alessia Ligresti

Mario Campanile
Molecular underpinnings of neural differentiation involved in the pathogenesis of glioblastoma
Tutor: Prof. Generoso Luca Colucci D'Amato

Giorgio Amendola
Application of Advanced In Silico Tools in the Design of Bioactive Small Molecules
Tutor: Sandro Cosconati

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

Gemma Conte
Biodegradable nanoparticles for prolonged therapeutic efficacy of antimicrobial peptides against Pseudomonas aeruginosa lung infections
Tutor: Ivana D'Angelo

Grant Garren January
Exploitation of new strains for drug discovery from Antarctic deep-sea sediments
Tutor: Dr. Donatella de Pascale

Janardhan Ausuri
Isolation and Characterization of Hydrocarbon degrading microorganisms
Tutor: Dr. Donatella De Pascale

Michele Minopoli
Structure-function relationship of an Urokinase receptor-derived peptide which prevents migration/invasion and vascular infiltration of cancer cells by inhibiting the formyl peptide receptor type 1 activity.
Tutor: Maria Vincenza Carriero

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

Viera Laura Santana
Targeting of an epigenetic regulator complex by a novel aptamer-based strategy
Tutor: Paola Ungaro

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

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

Narender Kumar
A cell-autonomous PD-1/PD-L1/2 circuit promotes proliferation and motility of thyroid cancer cells by potentiating the Ras/Mapk signalling cascade
Tutor: Prof. Rosa Marina Melillo

Henu Kumar Verma
Functional characterization and regulation of genes involved in the early phases of embryonic development
Tutor: Prof. Geppino Falco

Chetan Bharatkumar Dhakan
Evaluating MRI-CEST imaging of cancer metabolism and acidosis for characterizing murine tumor aggressiveness.
Tutor: Prof. Silvio Aime

Alejandro Moreiras Figueruelo
Drug-discovery from marine natural compounds
Tutor: Prof. Angelo Fontana; mail: afontana@icb.cnr.it

Gene Regulation and Computational Biology

Ankit Verma
Differential methylation profile in congenital imprinting disorders with multi-locus imprinting disturbances
Tutor: Andrea Riccio

Basilia Acurzio
Imprinting Control Regions binding proteins: key factors for maintaining genomic imprinting in mouse embryonic stem cells.
Tutor: Andrea Riccio

Ichcha Manipur
Metabolic network classification and Integrated analysis of multi-omics single cell sequencing data
Tutor: Mario Rosario Guarracino

Monika Krzak
Cell subpopulation detection through clustering single-cell RNAseq data
Tutor: Claudia Angelini

Carlo Giaccari
The role of NLRP5 in genomic imprinting. A new mutant mouse model
Tutor: Andrea Riccio

Amarinder Singh Thind
Pipelines and software tools for transcriptomics data integration and analysis uncover new biological insights
Tutor: Mario Rosario Guarracino

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: 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
Ostreatin, the second member of ribotoxin-like proteins from the edible mushroom Pleurotus ostreatus: purification and enzymatic characterization.
Tutor: Antimo Di Maro

Mariangela Valletta
Uncovering the molecular basis of cancer by cutting-edges high resolution mass spectrometry technologies
Tutor: Prof. Alessandro Usiello; Dr. Massimo Carella

Michela Napolitano
Mechanisms of CtBP1-S/BARS-mediated mitotic Golgi fragmentation
Tutor: Carmen Valente; Anna Chiara De Luca

Marica Sassano
NGF (1-14) peptide interaction with metal ions. NMR studies on structural changes.
Tutor: Gaetano Malgieri

Teresa Maria Carusone
Posttranscriptional mechanisms that impact on the regulation of expression and activity of the human lactonase PON2
Tutor: Giuseppe Manco

Andrea Corvino
Structure and dynamics of Human Prion and Phox2B proteins by Nuclear Magnetic Resonance (NMR)
Tutor: Roberto Fattorusso

Giuseppina Crescente
LC-MSn-based (poly)phenol profiling of extracts from food by-products with nutraceutical and cosmeceutical value
Tutor: Severina Pacifico

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

Daniela Caruso
Structural characterization of the aggregates formed by the members of the GADD45 family
Tutor: Menotti Ruvo and Luigi Vitagliano

Francesca Della Sala
Polymeric biomaterials and stem cells for pulmonary tissue regeneration.
Tutor: Assunta Borzacchiello

Alessandra Monti
Identification of inhibitors of PIN1 isomerase activity.
Tutor: Nunzianna Doti

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

Giovanni Mastroianni
Untargeted Metabolomics evaluation of nutraceuticals using NMR as main analytical platform
Tutor: Antonio Fiorentino

Alessia Casamassa
Investigating the metabolism of the D-amino acids, D-serine and D-aspartate, in the serum and CSF of patients with neurological disorders
Tutor: Prof. Alessandro Usiello; Dr. Massimo Carella

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

Rinaldo Grazioso
The effects of the metal ion replacement on the folding mechanism in the prokaryotic zinc-finger domain Ros87.
Tutor: Carla Isernia

Joëlle Ayoub
Insights into the tumor associated protein P150,
the largest subunit of the Chromatin Assembly Factor 1
Tutor: Giuseppina De Simone and Simona Maria Monti

Molecular Cell Biology

Alba Clara Fernández-Rilo
Untangling the phosphorylation of tau by understanding the role of novel interplayers: Endocannabinoid system, Orexin, Leptin and LPA.
Tutor:Luigia Cristino

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

Giuseppe Delli Paoli
A combination of exercise with fasting leads to a boost of fatty acid consumption in rats and humans, with beneficial outcomes.
Tutor:Pieter de Lange

Federica di Giacomo Russo
D-Aspartate in the 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

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

Giuseppe Petito
3,5 diiodo-L-thyronine (T2) improves the inflammatory response in visceral white adipose tissue (VAT) of rats fed a high fat diet
Tutor: Antonia Lanni

Concetta Iovine
In vivo and *in vitro* evaluation of ellagic acid effects on human and animal reproduction
Tutor: Lucia Rocco

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

Namrata Iyengar
Delineating cargo classes and their differential requirement of Signalling cascade for exit from the Endoplasmic Reticulum (ER)
Tutor: Dr. Alberto Luini

Stefania Serpico
The Lysophosphatidic Acid Acyltransferase (LPAATs) Enzymes and their tole in membrane transport alterations in cancer.
Tutor: Carmen Valente

Manpreet Patheja
Characterization of the Cellular targets of the Glycerophosphoinositols
Tutor: Alessia Varone and Daniela Corda

Human genetics

Ahmed El-Sharkawy 14:20 - 14:35

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

Rosita Del Prete 14:35 - 14:50

Characterization of murine models in imprinting disorders
Tutor: Alfonso Baldi

Jamal Naderi 14:50 - 15:05

Profiling histone modification (H3K4me3) in preadipocytes of individuals with a family history of type 2 diabetes
Tutor: Claudia Miele

Laura Pignata 15:05 - 15:20

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

Antonella Rendina 15:20 - 15:40

Neuroimmune mutations leading to dementia: focusing on microglial receptor CD33
Tutor: Emilia Vitale

Lucia Verrillo 15:40 - 15:55

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

Salvatore Fioriniello 15:55 - 16:15

Transcriptional and epigenetic deregulation of glycosphingolipid metabolism in Rett syndrome models
Tutor: Floriana Della Ragione

Domenico Marano 16:15 - 16:25

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 16:25 - 16:40

Identification of genomic variants responsible for pregnancy loss
Tutor: Vincenza Colonna

Paola D'Ambrosio 16:40 - 16:50

Therapeutic approach with Ataluren in Duchenne symptomatic carriers with nonsense mutations in dystrophin gene.
Tutor: Luisa Politano

Hilal Kalkan 16:50 - 17:00

SQSTM1/p62 Bridges Paget Disease of Bone to Neurological Disorders
Tutor: Gianfrancesco Fernando

Morone Barbara 17:00 - 17:10

Uncovering of ICF syndrome phenotypes in hematopoietic differentiation derivatives from patient- and gene corrected-iPSCs
Tutor: Maria R. Matarazzo

Maria Elena Onore 17:10 - 17:20

A new strategy for phase specific mutation detection
Tutor: Vincenzo Nigro

Session 1:

Cancer biology, Immunology, Microbiology, Drug design

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

PhD student: Alessia Ametrano
Tutor: Maria Rosaria Coscia, e-mail: mr.coscia@ibp.cnr.it
PhD cycle: 32° cycle
Affiliation: Institute of Biochemistry and Cell Biology National Research Council, Via Pietro Castellino, 111, 80131, Italy; Department of Enviromental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), University of Campania “Luigi Vanvitelli”, Caserta, Italy

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 mRuby, as selection marker for the correct sequence insertion, has been electroporated in c-myc hybridoma cells and I have obtained about 1% mRuby positive cells.

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

Modulating innate memory to treat inflammatory diseases

PhD student: Ana Margarida Ferreira Campos

Tutor: Paola Italiani (p.italiani@ibp.cnr.it)

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. 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 the role of GPI in innate memory will support the possibility of its pharmacological exploitation as an anti-inflammatory and memory-modulating drug to treat chronic inflammatory and autoimmune conditions. The previous employed methods to detect and quantify GroPIns can not be used in human primary cells (7), and are not applicable to supernatants (8). An analytical method to quantify GroPIns in both lysate and supernatant of cells was developed and applied to LPS-stimulated primary human monocytes, showing a gradual increase of intracellular GroPIns while the opposite was observed in the extracellular levels. These preliminary results further suggest the importance of GroPIns in inflammation and allow to further disclose GroPIns role.

1. Kumagai Y, Akira S. Identification and functions of pattern-recognition receptors. J Allergy Clin Immunol. 2010;125(5):985–92.
2. Bowdish DME, Loffredo MS, Mukhopadhyay S, Mantovani A, Gordon S. Macrophage receptors implicated in the “adaptive” form of innate immunity. Microbes Infect. 2007;9(14–15):1680–7.
3. Burch RM, Jelsema C, Axelrod J. Cholera toxin and pertussis toxin stimulate prostaglandin E2 synthesis in a murine macrophage cell line. J Pharmacol Exp Ther. 1988;244(2):765–73.
4. Gandhi CR, Hanahan DJ, Olson MS. Two distinct pathways of platelet-activating factor-induced hydrolysis of phosphoinositides in primary cultures of rat Kupffer cells. J Biol Chem. 1990;265(30):18234–41.
5. Gandhi CR, Stephenson K, Olson MS. A comparative study of endothelin- and platelet-activating-factor-mediated signal transduction and prostaglandin synthesis in rat Kupffer cells. Biochem J. 1992;281(Pt 2):485–92.
6. Vessichelli M, Mariggiò S, Varone A, Zizza P, Di Santo A, Amore C, et al. The natural phosphoinositide derivative glycerophosphoinositol inhibits the lipopolysaccharide-induced inflammatory and thrombotic responses. J Biol Chem. 2017;292(31):12828–41.
7. Berrie CP, Iurisci C, Piccolo E, Bagnati R, Corda D. Analysis of Phosphoinositides and Their Aqueous Metabolites. Methods Enzymol. 2007;434(07):187–232.
8. Grauso L, Mariggiò S, Corda D, Fontana A, Cutignano A. An improved UPLC-MS/MS platform for quantitative analysis of glycerophosphoinositol in mammalian cells. PLoS One. 2015;10(4):1–11.

Characterization of an efflux pump in *Mycobacterium smegmatis.*

PhD student: Barbara De Siena
Tutor: Lidia Muscariello (lidia.muscariello@unicampania.it)

PhD cycle: 32° cycle

Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), Università degli Studi della Campania Luigi Vanvitelli, Via Vivaldi 43, Caserta.

Tuberculosis is one of the top 10 causes of death worldwide. In 2017, 10 million people fell ill with TB, and 1.6 million died from the disease (WHO, *September* 2018). The ability of *Mycobacterium tuberculosis,* the causative agent of TB*,* to respond and adapt to various stresses is critical for the persistence of this human pathogen inside the host. We have previously described the role of a TetR-like protein of *M. smegmatis* and *M. tuberculosis* in regulation of the *MSMEG_3762/63/65* and *Rv1687c/86c/85c* operons (Perrone *et al*., 2017). In *M. smegmatis*, *MSMEG_3762* and *MSMEG_3763* are annotated as ABC transporter ATP-binding protein and as a transmembrane protein, respectively, as well as their orthologues in *M. tuberculosis*. The deduced aa sequences of the two orthologue operons share 74% identity. In order to define the role of this efflux system, I isolated a strain carrying a deletion in *MSMEG_3763*. A comparative analysis between the *M. smegmatis* wt and the *MSMEG_3763* deletion mutant suggest the involvement of this efflux pump in biofilm formation and extrusion of some first- and second-line anti-TB drugs.
Transcriptional analysis of the efflux pump operons in *M. smegmatis and M. tuberculosis*, during biofilm development and after macrophage infection, is under investigation.

Study of inflammatory response and complement activation following the use of engineered nanoparticles (ENPs).

PhD student: Giacomo Della Camera

Tutor: Dr. Diana Boraschi (d.boraschi@ibp.cnr.it)

PhD cycle: 33° cycle

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

The innate immune system must protect against diseases by preventing interaction with or invasion of virus, bacteria, cancer cells, and "not-self", maintaining the integrity of organs and systems in the body. On this regard, engineered nanoparticles (ENPs) represent important stimuli for innate immune cells, such as resident macrophages and circulating monocytes, which are responsible for the acute phase of inflammation. We have explored the effect of ENPs in the interaction with these cells by measuring the production of inflammatory interleukins and complement activation fragments in several biological matrices. Thus, based on our experience, we have selected, synthetized, and characterized some ENPs with interesting composition, size, shape, and surface functionalization. Furthermore, we have applied models of *in vitro* cell culture, in which monocytes and macrophages are exposed to selected ENPs, under specific experimental conditions (e.g. temperature, CO_2, and protein signals), to study the mechanisms involved in the "innate immune memory". Preliminary data indicate that composition and surface functionalization of ENPs, as well as their state of aggregation, shape, and endotoxin contamination, may potentially induce an immune response. Therefore, several aspects have to be considered during the ENPs' preparation because of their unknown side effects on human immune system.

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 is an aggressive malignancy with a high mortality rate that continues to be challenging to treat.
Repurposing the vast arsenal of non-oncology already-approved drugs, might be an attractive strategy to offer more-effective treatment options.
Increased expression of the mevalonate pathway and alteration of histone deacetylases (HDAC) are common aberrations found in many cancers such as pancreatic cancer.
We analyzed the antitumor effect of valproic acid (VPA), an anticonvulsant with HDAC inhibitory activity, and simvastatin (SIM), a cholesterol-lowering drug that inhibits mevalonate pathway, on pancreatic cancer cell lines ASPC1, MDA-Panc 28 and PANC1.
Our data showed a strong synergistic effect of valproic acid and statins, assessed by calculating the combination index according to Chou and Talalay method, on all cell lines. Moreover, clonogenic assay not only confirmed the synergistic effect of VPA/SIM but also revealed that the combination treatment was effective at very low doses.
To better recapitulate the complexity of pancreatic cancer we tested the combination on 3D in vitro microtissues of pancreatic carcinoma obtained by co-culturing pancreatic cancer cells and normal fibroblasts. A clear synergistic effect of VPA/SIM combination was confirmed with 3D Cell Viability already when we combined the two drugs at low doses.
Similar synergism were obtained when we combined VPA with different statins such as Atorvastatin and Lovastatin while the SIM/VPA combination is more effective than the combinations of SIM with other HDAC inhibitors.
Ongoing experiments are evaluating the combinatory approach based on the association of VPA/SIM with chemotherapy in vitro and in vivo and the molecular pathway underling this synergism.

Small molecules: towards a personalized therapy for Fabry Disease

PhD student: Ludovica Liguori

Tutor: Giuseppina Andreotti (giuseppina.andreotti@icb.cnr.it)

PhD cycle: 32° cycle

Affiliation: Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche. Comprensorio Olivetti, Edificio 70, Via Campi Flegrei 34 I-80078 Pozzuoli (Napoli).

Fabry Disease (FD) is a rare genetic disease caused by mutations in *GLA* gene that encodes for the lysosomal enzyme α-Galactosidase A (AGAL). There exist more than 700 missense mutations of the gene causing the disease with a large phenotypic spectrum. Therefore, the diagnosis is difficult, it is not possible to treat each patient in the same way and personalized therapy is required. There is a new therapeutic approach based on the pharmacological chaperone called DGJ, a small molecule that binds specifically AGAL stabilizing it and improving its expression and enzymatic activity within the cell. Nevertheless, DGJ is not the perfect molecule to treat Fabry disease, first because it is ineffective on some mutations and also because it is a strong inhibitor of AGAL. For this reason, we focused our study on the screening of new small molecules that can rescue AGAL activity without inhibiting it acting directly or indirectly on the enzyme. Moreover, we developed a cellular model constituted by patients fibroblasts stably transfected with *GLA* mutants to test substances. In stable transfections, there is not an abnormal overproduction of proteins so we think that this model could better represent what happens in cells when a substance acts indirectly on protein folding.

A proteomic approach identified HSP90 as a central hub in CDDP-resistant ovarian cancer cells: role of HSP90 inhibitors in overcoming drug resistance

PhD student: Rita Lombardi,

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

PhD cycle: 33° cycle

Affiliation: STITUTO NAZIONALE TUMORI – IRCCS – FONDAZIONE G. PASCALE/ CROM, MERCOGLIANO (AV)

Epithelial ovarian cancer (EOC) is the fourth leading cause of cancer death in women. Standard care for these patients combines radical surgery with platinum-taxol chemotherapy. The development of a platinum resistant disease is a frequent event and predicts poor prognosis. On this regard, we have generated and characterized three cisplatin (CDDP)-resistant isogenic high grade EOC cell lines (TOV112D Mi-res, OVSAHO Mi-res and MDAH MI-res) from their parental counterpart. First, by a proteomic approach we compared the protein expression profile of the three CDDP-resistant models and parental cells. To recognize the regulatory pathways in which the identified proteins were involved and hence to elucidate their biological functions and relationship with the cisplatin resistance, we interrogated the Ingenuity Pathway Analysis software. The protein chaperone HSP90 emerged as a main central hub and its up-regulation was observed in the CDDP-resistant cells. Since HSP90 plays a critical role in malignant transformation, we evaluated the effect of CDDP in combination with two HSP90 inhibitors: 17-AAG and GANETESPIB, both of them in phase II/III in cancers patients, demonstrating a *strong anti-proliferative effect* in EOC MI-res cells according to Chou and Talalay method and confirmed by the colony formation assay. Moreover, we also observed a *strong synergistic pro-apoptotic effect* in resistant ovarian cancer cells. Overall, our data demonstrated a potential role of HSP90 inhibitors as an innovative antitumor strategy that warrants further clinical evaluation.

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

PhD student: Alessandro Verde

Tutor: Dott.ssa Anna Chiara De Luca (a.deluca@ibp.cnr.it); Dott.ssa Paola Italiani (p.italiani@ibp.cnr.it)

PhD cycle: 34° cycle

Affiliation: CNR – Istituto di Biochimica e Biologia Cellulare (IBBC)

Bacterial Endotoxin, also known as Lipopolysaccharide (LPS), is a biological component that can be found on cell walls of gram-negative bacteria and it is considered one of the major contaminants. The presence of LPS on pharmaceutical and biomedical devices can be responsible for many inflammatory effects causing an immune activation of signalling pathways and therefore affecting the NPs toxicology properties. The aim of this work is to study the interaction of gold nanoparticles (Au NPs) with cells, accurately distinguishing the intrinsic NPs biological effects from those induced by LPS. The effect of LPS absorption on Au NPs and the formation of a biocorona will be characterized by means of imaging and spectroscopic techniques. Au NPs with sizes ranging from 25 to 50 nm will be exposed to E. coli LPS under different conditions to study the dose and time-dependent bind ability of the LPS. The internalization kinetic of bare Au NPs and LPS-coated Au NPs will be analysed in macrophages differentiated form peripheral blood monocyte cells (PBMC) and in cells by TEM and Raman imaging. Finally, the inflammatory response of bare and LPS-coated Au NPs will be analysed and compared through ELISA test to quantify cytokines production related to inflammation-inducing activities.

New Players involved in High Grade Serous Ovarian Carcinoma originating from Fallopian tube

PhD student: Priyanka Gokulnath

Tutor: Dr. Mariastella Zannini (s.zannini@ieos.cnr.it)

PhD cycle: 32nd cycle

Affiliation: Istituto per l'Endocrinologia e l'Oncologia Sperimentale 'G. Salvatore'

High Grade Serous Ovarian Carcinoma (HGSC) is one of the most aggressive and lethal gynecological malignancies attributed by its very late diagnosis and absence of early stage markers. Recently, the origin of HGSC has been ascribed to transformation events occurring in the Fallopian tube epithelium (FTE) in particular in the secretory cells of the FTE. PAX8, a member of the *Paired box* gene family of transcription factors required for the development and maintenance of specific organs, is an important histological marker of HGSC and also the lineage-specific marker of secretory cells in FTE. To understand the progression from FTE to HGSC and the possible role of PAX8 in this process, our laboratory performed RNA-sequencing analysis of FT-194 (FTE secretory cell line) and SKOV-3 (Ovarian cancer cell line) before and after PAX8 silencing. The results from the RNA-sequencing analysis suggested certain pathways to be regulated by PAX8 and also gave a list of dysregulated transcripts including several non-coding RNAs that were possibly involved in tumor progression from FT to Ovarian cancer. We reasoned that the role of PAX8 should be explored as this might be crucial in understanding the progression to HGSC and this was pursued by analyzing the physiology of FTE *in vivo* and *in vitro* using various molecular techniques. In parallel, we also focused on non-coding RNAs that are now believed to be master regulators of major cellular processes and in disease due to their ability of epigenetic, transcriptional, post- transcriptional and post-translational regulation. We have identified two lncRNAs potentially involved in ovarian tumor progression, MAGI2-AS3 and HAND2-AS1, by bioinformatically analyzing various databases and datasets and corelating with our studies. We experimentally validated these results in a panel of HGSC cell lines and further unraveled tumor suppressive role of these lncRNAs in HGSC using a competing endogenous network.

Therapeutic Potential of a Novel $\alpha_v\beta_3$ Antagonist to Hamper the Aggressiveness of Mesenchymal Triple Negative Breast Cancer Sub-Type

PhD student: Billy Samuel Hill

Tutor: Dr Antonella Zannetti

PhD cycle: 32° cycle

Affiliation: IBB-CNR

The mesenchymal sub-type of triple negative breast cancer (MES-TNBC) has a highly aggressive behavior and worse prognosis, due to its invasive and stem-like features, that correlate with metastatic dissemination and resistance to therapies. Furthermore, MES-TNBC is characterized by the expression of molecular markers related to the epithelial-to-mesenchymal transition (EMT) program and cancer stem cells (CSCs). The altered expression of $\alpha_v\beta_3$ integrin has been well established as a driver of cancer progression, stemness, and metastasis. Here, we showed that the high levels of $\alpha_v\beta_3$ are associated with MES-TNBC and therefore exploited the possibility to target this integrin to reduce the aggressiveness of this carcinoma. To this aim, MES-TNBC cells were treated with a novel peptide, named ψRGDechi, that we recently developed and characterized for its ability to selectively bind and inhibit $\alpha_v\beta_3$ integrin. Notably, ψRGDechi was able to hamper adhesion, migration, and invasion of MES-TNBC cells, as well as the capability of these cells to form vascular-like structures and mammospheres. In addition, this peptide reversed EMT program inhibits mesenchymal markers. These findings show that targeting $\alpha_v\beta_3$ integrin by ψRGDechi, it is possible to inhibit some of the malignant properties of MES-TNBC phenotype.

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

PhD student: PIETRO IRRERA

Tutor: Dario Longo (dario.longo@unito.it)

PhD cycle: 33° cycle

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Abstract congressi

EMIM 2015 – (European Molecular Imaging Meeting), Tubingen, Germany - "Noninvasive evaluation of renal pH homeostasis after ischemia reperfusion injury by CEST-MRI"

Functional Renal Imaging 2017 – 2nd International Scientific Symposium, Berlin (Germany) 11-13 October 2017 – "Noninvasive evaluation of renal pH homeostasis after ischemia reperfusion injury by CEST-MRI pH mapping"

Functional Renal Imaging 2017 – 2nd International Scientific Symposium, Berlin (Germany) 11-13 October 2017 – "Simultaneous assessment of kidney perfusion and pH in an acute kidney injury murine model exploiting a dynamic CEST-MRI approach"

EMIM 2019 – (European Molecular Imaging Meeting), Glasgow, Scotland – "An optimized multislice sequence for 3D MRI-CEST pH imaging"

ESMRMB 2019 – (The European Society for Magnetic Resonance in Medicine and Biology) "An Optimized Multislice Sequence for 3D MRI-CEST Imaging"

Functional Renal Imaging 2019 – 3rd International Scientific Symposium, Nottingham, UK 15-17 October 2019 – ""Mapping the dysregulation of renal acid-base homeostasis upon sepsis-induced shock by CEST-MRI"

Fibril formation assay to test new halogenated compounds as transthyretin misfolding inhibitors

PhD Student: Mariateresa Allocca

Tutor: Giuseppina Andreotti (gandreotti@icb.cnr.it)

PhD cycle: 34°cycle.

Affiliation: Istituto di Chimica Biomolecolare (ICB) CNR, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy. Biomolecular Science,

Transthyretin (TTR) is a tetrameric protein whose misfolding leads to several diseases because of formation of insoluble fibrils. Some molecules can stabilize the protein and prevent TTR aggregation *in vitro* but an effective therapy is still lacking.

During this period, I performed experiments in order to assess the effect on fibril formation of halogenated compounds recently synthesized by P. Peluso (ICB- CNR, Sassari). Wild type (wt) and mutants TTR were prepared from an *E. coli* expression system inducing TTR synthesis by addition of 0.4 mM IPTG for 5 h. After enzymatic lysis, proteins were fractionated by ammonium sulfate precipitation (55- 85%), then by anion exchange chromatography on a Q-Sepharose column. Purest fractions were combined and dialyzed against 10 mM phosphate buffer pH 7.6 obtaining 50- 100 mg of purified wt protein per liter of culture.

wt- TTR was used to test the molecules through a turbidity assay. TTR (0.4 mg/ml) in phosphate buffer was incubated with the selected drug at 35.5°C for 30 min then was diluted 1:1 with 200 mM acetate buffer pH 4.2 to reach the desired acidic condition. The turbidity at 400 nm was measured after 72 h incubation at 35.5°C. Preliminary results show that five compounds inhibit TTR fibril formation to 17–56 % of the value observed in the absence of drug.

The Endocannabinoid System as a target for the treatment of bone disorders

PhD student: Magdalena Kostrzewa

Tutors: Dr. Fernando Gianfrancesco (fernando.gianfrancesco@igb.cnr.it) /
Dr. Alessia Ligresti (alessia.ligresti@icb.cnr.it)

PhD Cycle: 33° cycle

Affiliation: Institute of Genetic and Biophysics (IGB) / Institute of Biomolecular Chemistry (ICB), National Research Council (CNR).

Endocannabinoid system (ECS) is a complex cell-signaling system of endogenous mediators (endocannabinoids), membrane receptors and metabolizing enzymes. Recent studies suggest an important role for ECS in the regulation of skeletal remodeling and the consequent implications on bone mass and disorders including bone metastasis. Paget disease (PDB) is the second most common chronic metabolic disorder characterized by increased bone turnover and disorganized osteoclastic and osteoblastic activity. Recently, germline mutation (P937R) in the ZNF687 gene has been identified as genetic cause of Giant Cell Tumor associated with PDB (GCT/PDB)[1].

We aimed to investigate the involvement of ECS in PDB progression. To assess this goal, Zfp687 Knock-in mouse model has been developed. Moreover cellular models of RAW264.7 cells (pre-osteoclasts) and MC3T3-E1 (pre-osteoblasts) have been used. Generation of stable Zfp687 knock-out of both cell type is ongoing. Micro-CT analyses of femurs showed an impairment of structural parameters at 3-month with full-blown disease at 8-months. Preliminary data between femurs from 1 and 7 months old $Zfp687^{(-/-)}$ mice showed differences in EC levels among groups and further studies are ongoing. Robust modulation of ECS components have been found also in differentiating WT-MC3T3-E1 cells, highlighting the importance of the ECS bone mass regulation at cellular level.

Molecular underpinnings of neural differentiation involved in the pathogenesis of glioblastoma

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PhD cycle: 34° cycle

Affiliation: Cellular and molecular Neuropathology laboratory.

Glioblastoma (GBM) is the most malignant and the most frequent tumor of the central nervous system. The World Health Organization (WHO) classifies gliomas in levels from I to IV based on histopathological criteria and invasive capacity of brain parenchyma. The preferential localization of the tumor mass is in the cerebral hemispheres and is responsible for cognitive symptoms based on its location. The average survival of the patients is approximately 15 months from the diagnosis given that the risk of recurrence following surgical removal and treatment with chemotherapy in combination with radiotherapy is concrete. The molecular mechanisms underlying GBM are complex and a complete view of the ethiology of GBM is still missing. In facts the deregulation of numerous signaling pathways, the presence of the blood-brain barrier, which limits the possibilities for chemotherapeutic drugs to reach the tumor site, and the existence of a population of cells with stem-like properties responsible for the reappearance of the tumor following therapy, may affect the chemoresistance of GBM. Studying gene expression control and tumor transdifferentiation can represent an advantageous strategy for the discovery of key mechanisms and the development of new treatments that allow to prolong and improve patients' life conditions.

Application of Advanced In Silico Tools in the Design of Bioactive Small Molecules

PhD student: Giorgio Amendola

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PhD cycle: 32° cycle

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Today, the employment of in silico methods permeates all the phases of the drug discovery process. Indeed, the synergic combination of traditional medicinal chemistry strategies with advanced in silico methods can yield impactful scientific results. In particular, during my Ph.D. training, I could work on several research projects which led to the identification of a number of novel biologically active compounds. These studies were aimed at discovering potential agents to treat different kinds of human conditions, ranging from resistant cancer types to neglected diseases. Through state-of-the-art methods, like molecular docking and molecular dynamics simulations, I could study the binding modes of the different compounds. This has allowed to provide critical insight into the rationalization and optimization of their activity. To achieve this, elaborate technical solutions have been found and applied in challenging cases, such as the docking and the dynamic simulation of compounds that bind covalently to their target, or compounds that chelate a catalytic zinc ion in the binding site. To conclude, some of the active agents identified in these projects are now undergoing through the optimization phase, which is being carried out both via traditional binding assays and the cutting edge FEP computational method.

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

PhD student: Ali Mokhtar Mahmoud

Tutor: Dr. Alessia Ligresti (aligresti@icb.cnr.it)

PhD Cycle: INCIPIT Biomolecular sciences

Affiliation: Institute of Biomolecular Chemistry (ICB), National Research Council (CNR).

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.

Biodegradable nanoparticles for prolonged therapeutic efficacy of antimicrobial peptides against *Pseudomonas aeruginosa* lung infections

PhD Student: Gemma Conte

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PhD Cicle: 34° cycle

Affiliation: Di.S.T.A.Bi.F

Due to their excellent *in vitro* activity against multidrug resistant bacteria, antimicrobial peptides (AMPs) hold great promise for the treatment of *Pseudomonas aeruginosa* lung infections in cystic fibrosis (CF) sufferers. In this work, poly(lactide-co-glycolide) acid (PLGA) nanoparticles for lung delivery of the frog skin-derived AMPs Esc(1-21) or its diastereomer Esc(1-21)-1c were successfully developed. Improved peptide transport through artificial CF mucus and simulated bacterial extracellular matrix was achieved *in vitro*. The formulations could be effectively delivered through a liquid jet nebulizer already available to patients. Of note, peptide-loaded nanoparticles displayed a prolonged efficacy in inhibiting *Pseudomonas aeruginosa* growth *in vitro* and *in vivo*. A single intra-tracheal administration of Esc peptide-loaded nanoparticles in a mouse model of Pseudomonas *aeruginosa* lung infection resulted in 3-log reduction of pulmonary bacterial burden up to 36 h. Overall, results unravel the potential of PLGA nanoparticles as a reliable system for controlled release of AMPs at lungs.

Exploitation of new strains for drug discovery from Antarctic deep-sea sediments

PhD student: Grant Garren January

Tutor: Dr. Donatella de Pascale –(d.depascale@ibp.cnr.it)

PhD cycle: 32° cycle

Affiliation: Institute of Protein Biochemistry, CNR

Introduction Currently, multidrug resistant infections are a primary concern for the WHO and society as a whole. Microorganisms, such as human pathogenic bacteria have developed resistance to antibiotics primarily through the misuse and overuse of these drugs. Extreme environments such as the Antarctic, harbour a diverse range of microorganisms that have over the course of evolution developed molecular adaptations to these cold environments. The unique metabolism of these microorganisms includes secondary metabolites that have been reported to possess several bioactivities. In this PhD study, microbial diversity and metabolic potential of Antarctic deep-sea sediments are assessed for pharmaceutical and biotechnological applications. **Methods** Deep-sea sediments were sampled from 2000-5000 m from a previously unexplored environment: The South Shetland trough, Antarctica. Bacteria were isolated from sediments using culture-dependent techniques: longer incubation time, lower temperatures, and different culture media. Isolates were identified by 16S rDNA sequencing and 6 were considered interesting based on taxonomy. Three of these isolates were subjected to *de novo* whole genome sequencing, small-scale fermentations, and crude extractions using organics solvents. Extracts were assessed for anti-microbial activity using a liquid inhibition assay. **Results** 50 bacteria have been isolated using culture-dependent techniques. Three isolates were sequenced, assembled, and annotated. One species, W7, presents a new species and is being biologically and chemically characterized. **Conclusion** The genomes of 3 isolates have been mined for biosynthetic gene clusters or pathways. Using the bioinformatics genome data, compounds of interest are being expressed or activated *in vitro* using the OSMAC approach, which will be followed by chemical characterization (e.g. HPLC/MS/NMR) and toxicity evaluation (e.g. zebrafish system).

Isolation and Characterization of Hydrocarbon degrading microorganisms

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PhD cycle: 34° cycle

Affiliation: IBBC, CNR, Naples, Italy

University of Campania Luigi Vanvitelli

Polyaromatic hydrocarbons(PAH's) are ubiquitous environmental pollutants which enters the ecosystem due to improper disposal, spillage. Their low solubility in water makes them to treat with the current conventional methods. Microorganisms are proved to degrade PAH's when treated under different conditions. To understand the degrading potential of microbes, it is essential to study about bacterial isolates and the enzymatic pathway involved in degradation. Primary screening involves to identification of the potential microbes able to assimilate PAH's as carbon source and survive on the agar plates. This is clearly depicted by the formation of zone (clear zone method) around the microbial colonies. With the help of literature works and theoretical knowledge about the breakdown of PAH's, spectrometric techniques will help in understanding the reaction involved between the microbes and PAH's compounds. GC-MS, LC-MS are widely used mass spectrometric techniques to identify the daughter products formed out of the PAH's breakdown. The molecular analysis includes DNA extraction, PCR, TRFLP, 16S rRNA gene sequencing. By this, the genetic compartments involved in hydrocarbon degradation can be elucidated. Given the ubiquitous and toxic nature of the hydrocarbon pollutants, a multidisciplinary approach involving biological and chemical(analytical) are essential in achieving this goal.

STRUCTURE-FUNCTION RELATIONSHIP OF AN UROKINASE RECEPTOR-DERIVED PEPTIDE WHICH PREVENTS MIGRATION INVASION AND VASCULAR INFILTRATION OF CANCER CELLS BY INHIBITING THE FORMYL PEPTIDE RECEPTOR TYPE 1 ACTIVITY.

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The urokinase receptor of (uPAR) is a master regulator of cell migration and uPAR88-92 (SRSRY) is the minimal sequence required to induce cell motility by interacting with the formyl-peptide receptor type-1 (FPR1). By screening a library of SRSRY-derived peptides carrying amino-acid substitutions, the peptide Ac-(D)-Tyr-(D)-Arg-Aib-(D)-Arg-NH2 (RI-3) was identified as the best inhibitor of cell migration. RI-3 competes with SRSRY and N-formyl-methionyl-leucyl-phenylalanine (fMLF) peptides for binding to FPR1. Molecular dynamics and docking simulations of FPR1/fMLF, FPR1/SRSRY and FPR1/RI-3 complexes revealed that RI-3 shares the same binding site of fMLF and SRSRY on FPR1. However, while fMLF and SRSRY display the same agonist activation signature, translating binding into signaling, RI-3 does not interact with the activation region of FPR1, keeping receptor anchored to cell membrane and unable to activate signaling. RI-3 adopts the turn structure typical of the uPAR-FPR1 antagonists, is stable in human serum, reduces invasion and spreading of melanoma and sarcoma cells into organotypic co-cultures, inhibits trans-endothelial migration of sarcoma and melanoma cells, reducing vascular infiltration by sarcoma cell xenografts in nude mice. We propose RI-3 as new pharmacophore model for developing selective inhibitors of processes sustained by an excess of cell migration, such as chronic inflammatory and neoplastic diseases.

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

PhD student: Pranoy Sahu

Tutors: Prof. Alberto Luini, Dr. Riccardo Rizzo

PhD cycle: 33rd cycle

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Aberrant localisation, expression and function of different Golgi-complex glycosyltransferases are becoming more evident factors in tumor 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 the regulation of Golgi glycosyltransferases. Specifically, we found that GOLPH3 positively regulates glycosphingolipid (GSL) synthesis by controlling the localization and stability of B4GALT5, a Golgi glycosyltransferase. The change in sphingolipid metabolism induced by the manipulation of GOLPH3 levels has an impact on a signalling pathway responsible for cell proliferation, thus influencing cell growth.

Interestingly, we found that B4GALT5 (encodes for Lactosylceramide synthase enzyme), 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 ask whether B4GALT5 is an oncogene?

Gain-of-function experiments in immortalized mouse fibroblasts (NIH3T3) shows that B4GALT5 promotes cell growth and proliferation *in vitro.* Gene ablation studies shows that profound reduction in growth of cancer cells with B4GALT5 amplification. Mechanistically, B4GALT5 by altering the glycosphingolipid metabolism, specifically altering the globo-series of GSL affects the mTOR signalling in a phosphatidylinositol (3,4,5)-trisphosphate dependent manner and that the enzymatic activity is necessary for the growth.

Thus, genetic, functional and biochemical data suggest that B4GALT5 is a possible new oncogene in human cancer. Importantly, overall these data suggest that the inhibition of sphingolipid metabolism represents a valuable therapeutic option for cancer patients bearing the overexpression/ amplification of GOLPH3 or B4GALT5.

Targeting of an epigenetic regulator complex by a novel aptamer-based strategy

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Chromatin remodeling is considered to be one of the major systems that contribute to epigenetic alterations, a fact that has been associated with many human diseases, including cancer. Aberrant epigenetic modifications probably occur at an early stage of tumor development and are reversible. In this view, targeting chromatin remodeling pathways is currently evolving as a major therapeutic strategy in the treatment of several cancers.

The aim of this work is to address the epigenetic therapeutic targeting by developing a novel aptamer-based approach able to revert aberrant epigenetic modifications, thus limiting cancer progression. To address this issue we focused on blocking the formation of an oncogenic complex formed by the transcription factor SALL4 and the nucleosome remodelling deacetylase complex NURD.

SALL4 is down-regulated and absent in most adult tissues, however, is found to be restored 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.

We are addressing SALL4 targeting by making use of new promising molecules called aptamers: short synthetic nucleic acids that are selected for specific binding to target of interest. The strategy consists on the selection of new aptamers against SALL4 that can specifically disrupt the interaction by applying protein-SELEX (Systematic Evolution of Ligands by EXponential enrichment).

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 Toll-like receptors (TLR) 4 and TLR 2, which are known to be two key regulatory components of inflammation. Surprisingly, out of twenty only four were active for TLRs. Furthermore, only three fractions were selected for a further step of purification in order to obtain a single active class of molecule. Finally, the effectiveness in activating specifically TREM2 will be tested using *knockout* and/or *knockdown* cell lines and the mechanism of action of lead compounds will be further envisaged using microglial cell lines.

1. Heneka, M.T., et al. 2014. Innate immune activation in neurodegenerative disease, Nature Reviews Immunology **14**, 463–477.
2. Piccio L., et al. 2008. Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. Brain 131, 3081–3091.
3. Ulland T. K., et al. 2017. TREM2 maintains microglial metabolic fitness in Alzheimer's disease. Cell 170, 649.e13–663.e13.
4. Cutignano A., Nuzzo G., et al. 2015. Development and Application of a Novel SPE-Method for Bioassay-Guided Fractionation of Marine Extracts. Marine drugs vol. 13,9 5736-49. doi:10.3390/md13095736

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 of Ria Formosa's lagoon, Portugal, one of the most interesting was identified to be a *Vibrio sp.,* which showed a high 16S rRNA gene sequencing similarity with *Vibrio spartinae*. Spectrophotometric and HPLC-MS analysis revealed the presence of prodigiosin and cyclo prodigiosin as major metabolites, followed by many other peaks belonging to prodigiosin-like molecules.

The family of natural red pigments, called prodiginines (PGs), is characterised by a common pyrrolyl pyrromethene skeleton and a deep-red colour. Prodigiosin is the most known component of this family, its wide range of biological activities includes antimalarial, antifungal, immunosuppressant and antibiotic activities, but it has recently received renewed attention for its anticancer effect against many cancerous cell lines, showing a very low toxicity on the normal cell lines compared with the cancerous ones, this is the reason why the objective of this work is the further investigation of this particular family of natural products, going through the discovery of new prodigiosin-derivatives.

A cell-autonomous PD-1/PD-L1/2 circuit promotes proliferation and motility of thyroid cancer cells by potentiating the Ras/Mapk signalling cascade

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PhD cycle: 32° cycle

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Immunomodulatory molecules play critical roles in the regulation of immune responses. Immune checkpoints (IC), Programmed cell death-1 (PD-1) receptor and programmed cell death-ligand 1 and 2 (PD-L1, PD-L2), cause immunosuppression in cancer. PD-Ls of cancer cells bind to PD-1 on CD8+ T lymphocytes, and impair their functional activity leading to immunosuppression.

We observed that PD-1 and its ligands are expressed in thyroid carcinoma (TC) surgical samples and TC cell lines in culture, but not in normal thyroid, by IHC and mRNA analysis, respectively. PD-1 expression levels correlated with TC progression and malignant features. These findings suggest that human TC cells feature a PD-1/PD-L1 intrinsic circuit that may have a functional role.

PD-1 blockade with anti-PD1, -PD-L1, -PD-L2 siRNAs or neutralizing antibodies, significantly decreased the proliferation of TC cells by causing a G1 arrest in the cell cycle, without affecting apoptotic rate and vitality. Increased proliferation of TC cells was observed upon enforced expression of PD-1, or stimulation of PD-1 with soluble PD-L1. PD-1 expression and stimulation in TC cells increased the activity of the Ras/Raf/Mapk signalling cascade. PD-1 required the SHP2 phosphatase for its biological activities in TC cells. Inhibition of Erk signalling and cell proliferation was observed upon treatment with SHP2 siRNA or pharmacological inhibitors. Accordingly, PD-1 co-immunoprecipitated with SHP2, and translocation of SHP2 on the plasma-membrane was also observed upon enforced expression of PD-1 in TC cells. Enforced expression of PD-1 accelerated and PD-1 inhibition decreased the growth rate of TC cell xenografts in immunocompromised mice.

Our data demonstrate that inhibition of PD-1 circuit in TC may induce a dual effect by both re-establishing anticancer immunity, and impairing tumor growth by inhibiting the Ras/Raf/Mapk signalling pathway.

Functional characterization and regulation of genes involved in the early phases of embryonic development

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Introduction: Embryonic development is believed to involve an integrated set of complex and coordinated development of different tissues mediated by changes in the expression of many genes. However, our knowledge about the regulation of embryo development is still limited. Here, we want to analyze the role of *Duxbl1,* a protein belonging to the Dux family, that is the other member of *Dux*, it binds to *Zscan4* and negatively regulates it. So, because *Zscan4* is highly expressed at 2-cell stage, in embryonic developments our goal is to comprehend the role of *Duxbl1* on the zygotic gene activation, that is responsible for the destiny of the blastomeres, and analyze its effect on the differentiation. **Methods**: Plasmid Construction; pIres vector was obtained from a nearby lab. We cloned first GFP in this vector and then we cloned Flag-Duxbl1. We changed the CMV promoter with an EF1a promoter in order to have a stable expression of these proteins in the blastomeres. **Anticipated Results:** In Vitro Differentiation of ES Cells: Initially, we identified *Duxbl1* as a negative regulator of metastate induced by *Zscan4* upon retinoids treatment. Further, we are generating *Duxbl1* over-expressing strategy in which the gene expression will be promoted by elongation factor (EF1a) and it will be indirectly visualized through GFP reporter.

A multidisciplinary approach to explore the physiological effects of the "alien metabolite" caulerpin on zebrafish larvae

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PhD cycle: 32°cycle

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Green algae of the genus *Caulerpa* are invading vast areas in the Mediterranean and becoming an urgent issue. Besides the direct deleterious effect of invasive species, their characteristic bioactive compounds can critically affect native species. Indeed, the accumulation of the algal bis-indolic alkaloid caulerpin (CAU) in the tissues of the native fish *Diplodus sargus* eating on *Caulerpa cylindracea* correlates with metabolic disorders and lipid metabolism alterations through peroxisome proliferator-activated receptor (PPAR) activation. However, using a multidisciplinary "omics" approach we tried to understand its overall effects on lipid composition, metabolism and microbiota and their possible correlations. Some specificity of action led us to focus also in the ability of CAU to induce significant changes in the endocannabinoid system and affect the inflammatory response. Using the zebrafish larvae (*Danio rerio*) we are assessing variations in lipid and endocannabinoids levels by LC-MS, the inflammatory response by gene expression analysis while the microbiota has been characterized by Illumina sequencing and metabolomic studies through NMR spectrometry. Even though *C. taxifolia*, the so-called "killer alga", is also known to contain CAU, our chemical investigations led us to rule out its presence in *C. taxifolia* var. *distichophylla*, a strain recently found along the Sicilian coast.

Expression analysis of HLA class II alleles conferring genetic susceptibility to Celiac Disease

PhD student: Federica Farina

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PhD: 33° cycle

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Celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten in genetically predisposed individuals. Most of subjects affected by CD carry the Human Leucocytes Antigen (HLA) DQA1*05 and DQB1*02 predisposing alleles, located on chromosome 6 in *cis* or *trans* configurations. The expression of HLA class II predisposing genes by Antigen Presenting Cells (APC) contributes to the anti-gluten $CD4^+$ T cells immune response. Indeed, celiac patients show a higher expression of CD-associated DQA1*05 and DQB1*02 alleles compared to non-CD-associated ones, both in *cis* (DR1/DR3) and in *trans* configuration (DR5/DR7).

To investigate the molecular mechanism that controls the differential expression of risk alleles we analyzed the transcriptional regulation. Nascent RNA transcripts were captured through click chemistry and used as template for the cDNA synthesis to perform qRT-PCR using allele-specific primers. Preliminary results suggest that the high expression of CD-associated DQA1*05 and DQB1*02 risk alleles, respect to non-CD associated ones, is mainly determined by a difference of transcription rate.

RNA sequencing analysis has been performed on APC with DR5/DR7 genotype from affected and non-affected subjects. We found many differentially expressed genes belonging to several pathways that are up or down regulated in CD patients compared to the controls.

PhD Days 2019, XXXIII cycle:

Presentation title: Drug-discovery from marine natural compounds

Supervisor: Prof. Angelo Fontana; **mail**: afontana@icb.cnr.it

Student: Alejandro Moreiras Figueruelo; EU-H2020-MSC-ITN MarPipe.

Affiliation: Istituto di Chimica Biomolecolare (ICB) CNR Pozzuoli

Project abstract:

The aim of this research project is to identify novel bioactive compounds from marine organisms (protists and invertebrates) 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 related to 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. Further tests can be easily added for detecting other interesting biological activities and, from this point of view, the platform screen as proved to be suitable and robust (manuscript in preparation).

The following steps with the current data will concern an iterative bioassay-guided fractionation of active samples in order to identify active, possibly novel, chemical entities.

Attendance to congresses:

- **Poster**: **Authors**: Alejandro Moreiras-Figueruelo, Genoveffa Nuzzo, Clementina Sansone, Christian Galasso and Angelo Fontana. **Title**: Developing a Drug Discovery Platform applied to Marine Natural products. **Congress**: BIOPROSP_2019, Tromsø, Norway, 25-27 Feb. 2019.
- **Oral communication**: **Speaker**: Alejandro Moreiras Figueruelo. **Supervisor:** Angelo Fontana. **Title**: Drug-discovery from marine natural compounds. **Congress**: International Summer School in Natural Products (ISSNP) 2019. Naples and Maratea, Italy, 1-5 July 2019.
- **Poster**: **Authors**: Alejandro Moreiras-Figueruelo, Genoveffa Nuzzo, Clementina Sansone, Christian Galasso, Jeanette H. Andersen and Angelo Fontana. **Title**: Developing a Drug Discovery Platform applied to Marine Natural products. **Congress**: XVI International Symposium on Marine Natural Products (MaNaPro) and XI European Conference on Marine Natural Products (ECMNP), Peniche, Portugal, 1-5 Sept. 2019.

Experience abroad:

MARBIO laboratory, University of Tromsø under the supervision of Dr. Jeanette H. Andersen, from the 28 Feb to the 15 Apr 2019.

Session 2:

Gene Regulation and Computational Biology

Differential methylation profile in congenital imprinting disorders with multi-locus imprinting disturbances

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Congenital imprinting disorders arises due to defects in machinery responsible for maintenance of imprinted gene expression. Several congenital imprinting disorders have been reported in human till date, mostly with rare occurrence worldwide. Deleterious molecular lesions identified in imprinting disorders may accounts for distinct phenotypes and genome-wide differential methylation profile. In addition to primary loci, these perturbation(s) might affect other imprinted loci, which cause multi-locus imprinting disturbances (MLID). MLID has also been previously reported with genetic variants in maternal-effect genes, which are the part of subcortical maternal complex (SMC) of the human oocyte. Mutations in these maternal-effect genes known to cause recurrent pregnancy loss, abnormal embryonic development and altered methylation patterns at germline-derived differentially methylated regions (gDMRs). Recently, advanced genomics technology has been proven to be useful in identifying genetic and epigenetic defects in MLIDs at a genome-wide level.

In the current study, we investigated two probands diagnosed with rare form of two different imprinting disorders with MLIDs. Genotyping analysis revealed novel mutations in maternal-effect genes. Genome wide methylation profile using Methylation Array showed defects at multiple loci associated with differentially methylated regions (DMRs). Thus, genomics based approaches coupled with computational setups enables to efficiently study imprinting disorders with MLIDs, complement the clinical findings, allow better management of disease and plan subsequent pregnancies.

Imprinting Control Regions binding proteins:

key factors for maintaining genomic imprinting in mouse embryonic stem cells

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Imprinting Control Regions (ICRs) are genomic loci controlling the monoallelic and parent-of-origin specific expression of imprinted genes in placental mammals. Genomic imprinting is established at ICRs during gametogenesis by differential DNA methylation of paternal and maternal alleles. In humans, loss of DNA methylation at ICRs leads to severe developmental disorders. Recently, the KRAB-Zinc Finger proteins,
ZFP57 and ZFP445, have been shown to bind the ICRs and be required for maintenance of DNA methylation during embryogenesis. However, a systematic study of all the factors interacting with the methylated and non-methylated ICR alleles is lacking. To address this issue, we are using several approaches that should allow to identify novel players involved in imprinting maintenance. The first approach is based on the characterization of the protein interactome of ZFP57, that includes proteins associated with mRNA processing/splicing, chromatin organization, transcription and genome stability. The second one is based on the search for transcription factors binding ICRs in mESCs by bioinformatics approaches. Chromatin Immunoprecipitation of two pioneer factors, followed by sequencing and allele-specific analysis, shows a crucial restriction of these TFs by DNA methylation from binding the imprinted allele at ICRs. This project has the potential to increase our current understanding on the molecular mechanisms of imprinting maintenance in early development.

Metabolic network classification and Integrated analysis of multi-omics single cell sequencing data

PhD student: Ichcha Manipur

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With the ever-increasing trove of omics data, network based exploration of cellular molecular interactions is moving forward at a rapid pace. Metabolic pathways, protein-protein interactions and gene regulatory networks are some of the commonly studied biological networks.

Network comparison is an active area of research which involves the study of the extent of similarities between networks from different conditions. One of my research activities involves using distribution based network distances, for the classification and clustering of metabolic networks. In our approach, each network is represented using probability distribution matrices of topological properties like node distance distribution and transition matrices. Pairwise distances between the distribution matrices are computed in order to build a Gram matrix.

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 in order to reduce the 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 significant reduction in time.

I am also involved in the analysis of tumor cell heterogeneity using single cell sequencing data. In [3], we studied the role of alternative splicing in determining tumor heterogeneity in breast cancer samples. 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 better discriminate the tumor subtypes.

1. Granata I, Guarracino MR, Kalyagin VA, Maddalena L, Manipur I, Pardalos PM. Supervised Classification of Metabolic Networks. 2018 IEEE International Conference on Bioinformatics and Biomedicine (BIBM). 2018.
2. Granata I, Guarracino MR, Kalyagin VA, Maddalena L, Manipur I, Pardalos PM. Model simplification for supervised classification of metabolic networks. Annals of Mathematics and Artificial Intelligence. 2019.
3. Manipur I[1], Granata I[1], Guarracino MR. Exploiting single-cell RNA sequencing data to link alternative splicing and cancer heterogeneity: A computational approach. The International Journal of Biochemistry & Cell Biology. 2019;108:51–60.

Cell subpopulation detection through clustering single-cell RNAseq data

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Single-cell RNA-seq (scRNAseq) is a powerful tool to study heterogeneity of cells. Recently, many computational tools have been proposed to identify distinct cell populations from scRNAseq data using clustering. Although clustering itself is a well established and well-known mathematical approach, single-cell data poses several challenges that make this task very difficult. As a consequence, novel tools emerge to overcome the inherent problems and provide more meaningful results. The general aim of my PhD project was to develop a new clustering algorithm that would allow for precise estimation of intracellular heterogeneity from scRNAseq data. The novel method, called ensMAP-DP, is based on probabilistic mixture modelling and constitute an optimal analysis pipeline for a variety of applications and research purposes. The second objective of my study, prior constructing the method, was to investigate the current practices in the analysis of scRNAseq data and to identify the major challenges in clustering. For this purpose, I performed an extensive comparative study evaluating the performance of several clustering methods on large scale real and simulated scRNAseq datasets. The results presented here provide important insights into the field of single-cell and can be useful to the general public to improve the methods and quality of their research.

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

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PhD cycle: 34° Cycle

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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 development. This phenomenon is called genomic imprinting. The genomic imprinting is regulated by epigenetic mechanisms as DNA methylation and histone modifications. These mechanisms, by acting differently between the two alleles in the Imprinting Control Regions (ICRs), modulate physical accessibility to DNA, regulating the expression of imprinted genes differently between the two allelic copies. In ICRs the DNA methylation is established during gametogenesis and protected from the genome-wide demethylation during the embryogenesis. The analysis of families with frequent abortions and Multi Locus Imprinted Disorder in offspring showed mothers with variants of NLRP5 gene. NLRP5 is a maternal effect gene, a category of genes that encode for mRNA or proteins accumulated in the cytoplasm oocyte during the oogenesis. They remain in the maternal cytoplasm, during the fertilization and in the early embryo stage, and influence the early embryonic development. Previous study in mouse demonstrated that the absence of Nlrp5 is deleterious for the embryo development. Through the generation of a new mutant mouse line we could well understand the relationship between Nlrp5 and genomic imprinting.

Pipelines and software tools for transcriptomics data integration and analysis uncover new biological insights

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Integrating data from various transcriptomics studies can help in hypothesising the biological state or phenotype characteristics of cellular systems. However, a comparison of high-throughput gene expression data-sets obtained from different experimental conditions is a challenging task. One way to overcome this challenge is to discover novel strategies and pipelines for data integration and interpretation.

The first part of my PhD work is defined in the following two sections: [a] Development of a web-based computational framework to compare gene expression signatures called RankerGUI. "RankerGUI", is a user-friendly web application designed to compare and characterise gene expression signatures from multiple platforms, different experimental conditions or both.[b] Development of the visualization architecture of the tool called Decontaminer. Tool can detect contaminating organisms in unmapped sequences. This visualization architecture of the tool provides summary statistics and interactive plots of the experiment results. Furthermore, web-based visualization version functionality allows users to search of interesting contaminants by selecting a subset of samples and setting up various thresholds.

The second part of my PhD was to investigate changes in gene expression levels of ageing/sexually maturing Paramecia. These analyses helped shed light on the molecular mechanisms that underlie sexual maturation in this single-celled eukaryote. This analysis helps further our understanding of the evolution of sex.

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 integrated analysis of Whole Genome Bisulfite Sequencing (WGBS), ChIP-Seq and RNA-Seq datasets from patient-derived iPSCs and their CRISPR/Cas9-corrected clones.

The Differentially Methylated Regions (DMR) located in the promoter and gene body were annotated and intersected with RNA-seq gene expression dataset to identify genes of interest. The identification of differentially enriched peaks from ChIP-seq analysis of DNMT3B and H3K4me3/H3K36me3 histone marks and their intersection with DMR-associated genes is currently ongoing.

Further studies will include motif analysis on ChIP-seq data, association of differential peaks to differentially expressed genes and finally integration of all datasets to identify gene targets and pathways that will help understand the development of molecular phenotypes of ICF syndrome and the DNMT3B role during early embryogenesis.

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

PhD student: Yi-Shin Lee,

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PhD cycle: INCIPIT 2017

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

Ostreatin, the second member of ribotoxin-like proteins from the edible mushroom *Pleurotus ostreatus*: purification and enzymatic characterization

PhD Student: Nicola Landi

Tutor: Antimo di Maro

PhD cycle: 33rd cycle

Affiliation: DISTABIF

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A specific ribonuclease, named Ageritin, from edible mushroom *Agrocybe aegerita*, which damage rRNA in the highly conserved α-sarcin/ricin stem-loop (SRL), as classic ribotoxins from ascomycetes, has been well characterized. Given its peculiar features, Ageritin is the prototype of a new ribonucleases class expressed in basidiomycetes. Consequently, our group is engaged in the research of novel members of this family from edible mushrooms, named "ribotoxin-like proteins".

Therefore, during the second year, I have characterize a second member of this family from *Pleurotus ostreatus* fruiting bodies, named Ostreatin. This enzyme inhibits protein synthesis with an IC_{50} of 234 pM, releases the α-fragment when incubated with either yeast or rabbit ribosomes, and is able to linearized supercoiled pUC18 DNA as occurred for Ageritin. Structurally, Ostreatin possesses a single free cysteinyl residue and shows a higher content in β-strand (~30%) with respect to α-helix (~10%).

Moreover, we determined the primary structure of Ageritin by using a strategy based on comparative mapping by MALDI-TOF MS and the screening of the *A. aegerita* genome. Subsequently a bioinformatics approach we identified the catalytic pocket in which three conserved residues, Asp68, Asp70, and His77 form the catalytic triad.

Uncovering the molecular basis of cancer by cutting-edges high resolution mass spectrometry technologies

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The complexity of cancer biology requires the collection of comprehensive information on tumor proteome. Advances in mass spectrometry (MS)-based proteomics technologies bridge the gap between disease phenotypes, genomics data and proteins, the major cellular players of cellular functions. The goals of MS approaches in cancer research are devoted to high-throughput mapping proteome changes into malignancy to identify protein signatures or biomarkers potentially useful for cancer diagnosis, prognosis and therapy.

The aim of this work is to set-up emerging high-resolution MS strategies, in conjunction with multiplexed immune assays, for in-depth protein profiling of selected tumor cancer cell models including triple-negative breast cancer (TNBC) and colon cancer (CRC).

Quantitative nano LC-MS/MS TMT isobaric labeling-based approaches have been applied to the investigation of molecular determinants of cancer resistance with a focus on Pentraxin 3 (PTX3) and Macrophage migration inhibitory factor (MIF).

By this strategy, we have delineated a molecular hallmark of cetuximab-resistance in CRC and identified MIF as a factor capable of triggering cancer resistance in sensitive CRC cells. Moreover, we collected preliminary results suggesting the association of PTX3 signaling and its dysregulation into TNBC, paving the way to further studies aimed at understanding the role of the PTX3 axis in breast cancer resistance.

Mechanisms of CtBP1-S/BARS-mediated mitotic Golgi fragmentation

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PhD cycle: 32° cycle

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The "Golgi mitotic checkpoint" is one the control mechanisms that regulate the correct cell cycle progression. At the onset of mitosis, the Golgi complex undergoes extensive fragmentation in two main sequential steps for its correct inheritance into the daughter cells and for mitotic entrance itself. The inhibition of the Golgi partitioning in living cells results in a G2 phase cell cycle arrest. The protein CtBP1-S/BARS (BARS) has been shown to mediate membrane fission during the mitotic Golgi ribbon unlinking as well as in several intracellular membrane transport pathways, including basolateral post-Golgi transport. At the Golgi membranes, BARS assembles into a complex where it binds to the phosphoinositide kinase PI4KIIIβ through a 14-3-3γ dimer, as well as to ARF and the PKD and PAK kinases. Once incorporated into this complex, BARS binds to and activates a Golgi-localized lysophosphatidic acid (LPA) acyltransferase type δ (LPAATδ). The LPAATδ enzyme catalyzes the conversion of LPA into phosphatidic acid (PA). This reaction is essential for membrane fission.

We aim to define the molecular mechanisms underlying the BARS-mediated mitotic Golgi ribbon cleavage. Our current work is aimed at detailed understanding of the role of the key BARS protein complex components as well as lipid components that regulate BARS-mediated fission during the G2/M transition.

NGF (1-14) peptide interaction with metal ions. NMR studies on structural changes.

PhD student: Marica Sassano

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PhD cycle: 34° cycle

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The influence of structural changes, folding/unfolding mechanisms of proteins/peptides induced by metal ions have been demonstrated for various molecules. (1, 2)
Among peptides, Nerve Growth Factor N-terminal (1-14) was widely investigated for the capability to mimic the whole protein. Moreover, has been demonstrated that NGF (1-14) interaction with copper(II) and zinc(II) is able to modulate its activity. (3)
Therefore the identification of other metal ions capable to inhibit or enhance the activities of NGF (1-14) peptide could be a promising tool for the development of good drug candidate for the treatment of several diseases in which the NGF signal transduction pathway is involved.
The project starts with peptide expression using the intein fusion system and a single purification step (4). The advantages of this cost-effective technique are either high yield peptide expression and easy purification, than the opportunity to introduce isotopyc labels (^{13}C, ^{15}N, ^{2}H) for NMR studies.
Metal ions able to interact with NGF (1-14) will be identified and conformational/structural changes studied by NMR. The following step should be the understanding of how these changes can influence the NGF (1-14) activity "*in vitro"* by cell-based assays.

1. Malgieri G., D'Abrosca G., Pirone L., Toto A., Palmieri M., Russo L., Sciacca M.F.M., Tatè R., Sivo V., Baglivo I., Majewska R., Coletta M., Pedone P.V., Isernia C., De Stefano M., Gianni S., Pedone E.M., Milardi D., Fattorusso R.
"*Folding mechanisms steer the amyloid fibril formation propensity of highly homologous proteins*"
Chem. Sci. 2918, **9**, 3290-3298.

2. Travaglia A., La Mendola D., Magri A., Pietropaolo A., Nicoletti V.G., Grasso G., Malgieri G., Fattorusso R., Isernia C., Rizzarelli E.
"*Zinc(II) interactions with Brain-Derived Neurotrophic Factor N-terminal peptide fragments: inorganic features and biological perspectives*"
Inorg. Chem.2013. 52, 11075-11083.

3. Pandini G., Satriano C., Pietropaolo A., Gianì F., Travaglia A., La Mendola D., Nicoletti V. G., Rizzarelli E.

"*The Inorganic Side of NGF: Copper(II) and Zinc(II) Affect the NGF Mimicking Signaling of the N-Terminus Peptides Encompassing the Recognition Domain of TrkA Receptor* "
Frontiers in Neuroscience, 2016 Dec. 20; **10**:569, 1-21.

4. Yong-Gang Xie, Fei-Fei Han, Chao Luan, et al.
"*High-Yield Soluble Expression and Simple Purification of the Antimicrobial Peptide OG2 Using the Intein System in Escherichia coli*"
BioMed Research International, vol. 2013, Article ID 754319.

Posttranscriptional mechanisms that impact on the regulation of expression and activity of the human lactonase PON2

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Paraoxonase 2 (PON2) is one of three highly conserved members of the paraoxonase family of enzymes comprising also PON1, and PON3. Among the PONs, PON2 shows the highest lactonase activity, and it is also an antioxidant and anti-apoptotic protein. Recent studies reported an association between increased PON2 protein level and some tumors. The onset and progression of cancer are affected by failures in regulatory mechanism of gene expression. Posttranscriptional events such as the control of mRNA degradation, stability, location, and translation, as well as protein post-translational modifications (PTM) are equally crucial and require sophisticated regulation by various intracellular signaling pathways. Recently we demonstrated that in vitro ubiquitination of Lys 144 was apparently responsible for PON2 inactivation. In this study we confirm this result in vivo, showing the occurrence of new PTMs of PON2 in HeLa cells. Some of them were found to gather nearby two SNPs: A144G and S311C, important for their association with diabetes and its complications. In vitro mutation analysis showed SNPs involvement in PON2 activity and suggested a role of PTMs on its modulation. In addition, we discovered a control of PON2 expression via a putative mRNA operon involving the Wilms tumour 1 associated protein (WTAP) and the E3-ubiquitin ligase baculoviral IAP repeat containing 3 (BIRC3).

Structure and dynamics of Human Prion and Phox2B proteins by Nuclear Magnetic Resonance (NMR)

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The focus of the PhD project is the structural and dynamics characterization of the human prion (HuPrp) and prion-like proteins using NMR. During the first year, my research activities have been focused on the description of the folding mechanisms of $HuPrp^{23-231}$ (full-length) and $HuPrp^{90-231}$ in the presence and 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 interact 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 prion-like proteins, I have focused my attention to a structurally analogous protein, Phox-2B, 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 and in the DNA-recognition mechanism, the structural characterization of Phox-2B containing the correct C-terminal (20 alanines) stretch by using NMR spectroscopy has been started. As a first step, according to the standard procedure defined by Wüthrich and co-workers, a nearly complete assignment of the backbone chemical shift has been obtained by using the standard triple resonance NMR experiments.

(1) S.B. Prusiner *Science* **252**, 1515-1522 (1991)
(2) Pattyn A, Morin X, Cremer H, Goridis C & Brunet JF (1999) The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399, 366–370.
(3) Pattyn A, Goridis C & Brunet JF (2000) Specification of the central noradrenergic phenotype by the homeobox gene Phox2b. Mol Cell Neurosci 15, 235–243.
(4) Stornetta RL, Moreira TS, Takakura AC, Kang BJ, Chang DA, West GH, Brunet JF, Mulkey DK, Bayliss DA & Guyenet PG (2006) Expression of Phox2b by brainstem neurons involved in chemosensory integration in the adult rat. J Neurosci 26, 10305–10314.

LC-MS^n-based (poly)phenol profiling of extracts from food by-products with nutraceutical and cosmeceutical value

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Food by-products and wastes, produced every day at early stages of the agri-food chain, are rich in valuable bioactive compounds, whose nutraceutical and cosmeceutical valorization could reach targeted applications. In fact, recovering waste materials is mandatory not only for reducing their environmental impact, and disposal costs, but also for converting them into highly qualified products. In this context, considering three different by-products and wastes (below listed), in the PhD research activity course, the recovery, and reuse of their (poly)phenol compounds were prompt to realize a virtuous system for achieving new sustainable and functional products. Thus, faulty zucchini fruits (*Cucurbita pepo* cv. 'Lungo Fiorentino'), intended for disposal, were rescued as inexpensive and bio-sustainable source for cosmeceutical purposes, whereas leaves of *Vitis vinifera* cv. Greco di Tufo, produced in large amounts by peeling, a practice commonly used with the aim of improving the harvest quality, were investigated as source of flavonol glycosides and glycuronides with neuroprotective effect and anti-acetylcholinesterase activity. Furthermore, (poly)phenols were obtained as complex mixture from oil-depauperated hemp seeds, an undervalued by-product in the actual *Cannabis* fruits revival. This (poly)phenol fraction, mainly consisted in phenylpropanoids amides and lignanamides, was evaluated for its cytotoxicity and genotoxicity towards human CNS cells.

Exciton and Charge Separation : Computational Models

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G-quadruplexes are four-stranded globular nucleic acid secondary structures formed in specific DNA G-rich sequences[1]. We are interested in studying the photodynamics of direct UV absorption of these biologically relevant species. Main players in the oxidative damage of GQ are Guanine radical cations that can be metastable towards the loss of a proton, either that bonded to N1 or N2 atom[2]. We have calculated the absorption spectra of isolated Guanine cation, G-H1 and G-H2 radicals using TD-DFT techniques and are in good agreement with experimental data. In order to understand the behavior of photodamaged GQ we are studying the human telomeric sequence GGG(TTAGGG) 3 (Tel21) in the presence of Na+ ions as the monovalent stabilizing cation. We have performed classical MD simulations of this sequence with and without the presence of Guanine cation with the aim of characterizing the conformational changes due to the presence of the positive charge. The MD studies is focused on structural features such as the stability of H-bonded G-tetrads, the conformational behavior of lateral and diagonal loops, the interactions with Na+ ions. These MD results can be integrated with TD-DFT calculations to predict CD spectrum and to interpret transient absorption experiments on these sequences.

References

[1] D. Rhodes and HJ. Lipps, Nucleic Acids Res. 2015, 43, 8627.

[2]L. Martínez-Fernández, A. Banyasz, D. Markovitsi, R. Improta, *Chem. Eur. J.* 2018, 24, 15185.

Structural characterization of the aggregates formed by the members of the GADD45 family

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PhD cycle: 32° cycle

The Growth Arrest and DNA damage-inducible 45 (GADD45) gene family encodes three related highly similar proteins: GADD45α, GADD45β, and GADD45γ [1]. These proteins are involved in fundamental physio-pathological processes which include growth arrest, cell cycle control, DNA repair and apoptosis. Recently it has been reported that the complex formed by the antiapoptotic factor GADD45β with the JNK kinase MKK7 represents an interesting therapeutic target in multiple myeloma [2]. During these investigations we found that GADD45β undergoes denaturation by forming β-rich amyloid-like aggregates that are cytotoxic in physiological conditions. The characterization of the unfolding process of the two other members of the family (GADD45α and GADD45γ) highlighted analogies and differences. Indeed, while GADD45α displays behaviour somehow similar to that exhibited by GADD45β, GADD45γ exhibits a partial and reversible unfolding without forming any aggregate. During the second year, further characterizations of these systems have provided insights into the determinants that favour/disfavour the amyloid-like aggregation in GADD45 proteins. Notably, we find that GADD45β amyloid–like aggregates are able to form non-toxic hydrogels and nanogels whose basic structural motifs are still the β-structure of amyloid-like systems. This year, using structural/biophysical techniques, limited proteolysis experiments, mass spectrometry and peptide synthesis, we have identified the protein region(s) potential involved in the self-aggregation events.

[1] R.E. Tamura, J.F. de Vasconcellos, D. Sarkar, T.A. Libermann, P.B. Fisher, L.F. Zerbini. *Curr Mol Med.* **2012**, *12*, 634.
[2] L. Tornatore et al. *Cancer Cell*, **2014**, 495.

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Polymeric biomaterials and stem cells for pulmonary tissue regeneration

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Lung diseases (LDs), both chronic and acute, are characterized by derangements of the alveolar walls and altered alveolar functions causing an impaired respiratory function. A common denominator of these diseases is the palliative effect of current therapies, leading to an aberrant repair process of lung tissue. Stem cells (SCs) therapy is an attractive approach to protect lung function, because it can simultaneously target multiple pathological processes. However, the anomalous remodeling of the extracellular matrix (ECM) occurring in LDs leads to a loss of lung scaffold precluding the normal tissue repair and the correct homing of SCs. In this frame, the aim of this research activity is to develop a novel regenerative therapeutic approach based on polymeric biomaterial scaffolds, mimicking the natural lung ECM and SCs, for the treatment of LDs. To this purpose, injectable polymeric scaffolds, liquids at room temperature and capable of assuming a viscoelastic consistency similar to that of the lung parenchyma at body temperature have been studied. The scaffold composition in terms of concentration and molecular weight has been investigated and the mechanical properties, the bio interaction and the capability of the scaffold to promote the differentiation of SCs in Type II Alveolar cells has been explored.

Identification of inhibitors of PIN1 isomerase activity.

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PIN1 is a peptidyl-prolyl cis-trans isomerase (PPIase)[1]. It isomerizes the phosphorylated Serine/Threonine-Proline bonds of many proteins, including kinases, transcription activators and regulators, altering their structures and activities[1-3]. Therefore, PIN1 plays a crucial role in many aspects of cellular functions, including cell survival and cell metabolism[3-4]. Noteworthy, PIN1 is over-expressed in several human tumors and many findings support the key role of PIN1 in tumorigenesis and tumor progression[3]. Noteworthy, the down-regulation of PIN1 induced by pharmacological treatment provides significant anti-tumor effects, demonstrating that inhibitors of PIN1 might offer a good approach for the treatment of tumors[5].

The aim of this study is the identification of new inhibitors of PIN1 isomerase activity. Starting from the natural substrates of PIN1, a library of new proline-containing tetrapeptides has been generated by molecular modeling and docking studies. The ability of each tetrapeptide to interact and inhibit PIN1, expressed and purified to homogeneity, has been analyzed by direct-binding assays by using Enspire-label free technique and by an optimized functional assay[6].

A set of peptides displays a good affinity to PIN1 and inhibits its isomerase activity. Data obtained provide a good starting point for the design of new molecules with improved affinity and selectivity to PIN1.

1. Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX. Nature (1989) 337, 476-8.
2. Göthel SF, Marahiel MA. Cell Mol Life Sci. (1999) 55, 423-36.
3. Lu Z, Hunter T. Cell Res. (2014) 24(9): 1033-1049.
4. Akihide Ryo, et al. J of Cell Science (2003) 116, 773-783.
5. Zheng M et al. Oncotarget. (2017) 8(18): 29771-29784.
6. Caporale A, et al. J Biomol Screen. (2016) 21, 701-12.

Secondary metabolites from Mediterranean plants for nutraceutical and pharmaceutical applications

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Modern pharmacological studies show that *Cistanche* genus have a broad spectrum of biological activities, attributed to the complex and varied phytochemical composition[1].
Being aware of the value of this genus, in this work we focus our attention on *Cistanche phelypaea*. Firstly, ethyl acetate, acetone, ethanol and water extracts from flowers, stems and roots of *C. phelypaea* (L.) Cout were appraised for radical scavenging activity. Furthermore, the most promising water extracts were evaluated for enzymatic inhibition related with the onset of acetylcholinesterase and butyrylcholinesterase, type 2 diabetes mellitus and skin hyperpigmentation (tyrosinase). Structure elucidation of water extracts were afforded by NMR (1D and 2D) analyses. The water extract of each section shows chemical differences: in stems, iridoids and phenylethanoids (PhGs) were detected, especially acteoside; in roots were detected essentially PhGs, mainly echinacoside and tubuloside A. The main identified compounds were tested for docking toward the selected enzymes used for the biological tests.
Finally, *C. phelypaea* may be a potential candidate for the treatment of various diseases as it represents a valuable source of bioactive compounds for pharmaceutical applications.

(1) Fu Z., Fan X., Wang X., Gao X. (2018) Cistanches Herba: An overview of its chemistry, pharmacology, and pharmacokinetics property. *Journal of Ethnopharmacology*, 219, 233-247

Untargeted Metabolomics evaluation of nutraceuticals using NMR as main analytical platform

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Interest in functional foods is constantly growing, nutraceuticals and other natural health products has been well recognized in relation with health promotion, disease risk reduction and reduction in health care costs. Metabolomics is a powerful approach because metabolites and their concentrations, unlike other "omics" measures, directly reflect the underlying biochemical activity and state of cells / tissues. In this project we concentrated on the study of metabolomics profiles of potentially nutraceutical products. We mainly focused our attention on several different cultivars of *Prunus persica* that have been analyzed through an NMR metabolomics approach which allows the quantification of the concentration and the study of the chemical structure of metabolites. The first part of research consisted in fine tuning the extraction system, we proceeded to set up and standardize a micro extractive biphasic system using $CDCl_3/CD_3OD/D_2O$ in order to extract secondary polar and lipophilic metabolites. All data obtained by ^1H NMR spectroscopy have been analyzed by multivariate statistics (PCA and PLS-DA). PCA scores plots were used to visualize differences between spectra and identify any correlation between cultivars. Additional activities in the research have been to test the *radical scavenging* of the extracts with various in vitro assays and the quantitation and identification of triterpenoids (steroids and pentacyclic triterpenes) by mass spectroscopy. Moreover, to define the structure of the metabolites responsible for the registered activities, an examination of more promising extracts have been done using 2D-NMR techniques.

Investigating the metabolism of the D-amino acids, D-serine and D-aspartate, in the serum and CSF of patients with neurological disorders

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D-aspartate and D-serine, act respectively as endogenous agonist and coagonist of NMDA glutamate receptors, modulating their functions in rodents and humans. In line with NMDA-hypofunction in schizophrenia, previous studies in the *post-mortem* brain showed that D-aspartate content was decreased by around 30-40% in patients. Based on these studies, I investigated whether the alterations of D-aspartate metabolism are selective for schizophrenia or are a common alteration of other neurological disorders such as Frontotemporal Dementia (FTD), Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), Parkinson's Disease (PD), Multiple Sclerosis (MS), Clinically Isolated Syndrome (CIS) and Radiologically Isolated Syndrome (RIS). In this regard, I analysed so far through an HPLC approach the D-aspartate and D-serine metabolism in the serum and the CSF of patients from 3 different Italian hospitals. Interestingly, my data showed an alteration in the CSF levels of D-/L-serine and L-glutamate in FTD, PD and SM patients, of L-aspartate in AD and RIS patients and of D-serine in CIS patients, but no main alterations of D-aspartate amount have been found. Overall these data suggest that variations in D-aspartate metabolism are specific to schizophrenia, thus suggesting a use for this amino acid as a possible biomarker in this devastating psychiatric disorder.

Targeting Nodal and Cripto-1 onco-fetal proteins using Bispecific antibody fragments

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Nodal is a potent embryonic morphogen belonging to the TGF-beta superfamily. Typically, it binds to Alk4/ActRIIB receptor complex in presence of the co-receptor Cripto-1. Expression of both Nodal and Cripto-1 is restricted to embryos and embryonic stem cells, whereas it's poorly present in normal adult tissues; their re-expression in adults is associated with many tumors, indicating them as diagnostic biomarkers and therapeutic targets for several cancers [1]. We have generated anti-Nodal and anti-Cripto-1 mAbs named 3D1 and 1B4, respectively. 3D1 therapeutic efficacy has been proven in aggressive melanoma also in vivo [2, 3]. We have produced partly humanized 3D1 and 1B4 Fabs to obtain new molecules with better PK/PD profiles. Through MTGase transglutamination reactions we have introduced fluorescent dyes useful for imaging applications. We present the biochemical characterization of 3D1 and 1B4 rFabs and the generation of bispecific Fab2 connected through linkers introduced using MTGase and having different length and flexibility to optimize recognition of the two antigens. These efforts have led bispecific Fabs of 3D1 and 1B4 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. Similarly, we are working at obtaining bispecific Fab2 useful in immunotherapy settings containing an anti-CD3 Fab (taken from the clinically approved Blinatumomab, [4-6]), and anti-Cripto and/or the anti-Nodal recombinant Fabs. The new molecules will be tested for their ability to simultaneously bind the two distinct antigens and characterized at structural level.

1. Bodenstine, T. M., Chandler, G. S., Seftor, R. E. B., Seftor, E. A., & Hendrix, M. J. C. (2016). Plasticity underlies tumor progression: role of Nodal signaling. Cancer and Metastasis Reviews, 35(1), 21–39.

2. Focà, A., Sanguigno, L., Focà, G., Strizzi, L., Iannitti, R., Palumbo, R., ... Sandomenico, A. (2015). New Anti-Nodal Monoclonal Antibodies Targeting the Nodal Pre-Helix Loop Involved in Cripto-1 Binding. International Journal of Molecular Sciences, 16(9).

3. Strizzi, L., Sandomenico, A., Margaryan, N. V., Focà, A., Sanguigno, L., Bodenstine, T. M., ... Hendrix, M. J. C. (2015). Effects of a novel Nodal-targeting monoclonal antibody in melanoma. Oncotarget, 6(33).

4. Wu, J.; Fu, J.; Zhang, M.; Liu, D., Blinatumomab: a bispecific T cell engager (BiTE) antibody against CD19/CD3 for refractory acute lymphoid leukemia. J Hematol Oncol, 2015, 8, 104.

5. Curran E, Stock W., Taking a "BiTE out of ALL": blinatumomab approval for MRD-positive ALL. Blood. 2019 Apr 18;133(16):1715-1719.

6. Jen EY et al., FDA Approval: Blinatumomab for Patients with B-cell Precursor Acute Lymphoblastic Leukemia in Morphologic Remission with Minimal Residual Disease. Clin Cancer Res. 2019 Jan 15;25(2):473-477.

The effects of the metal ion replacement on the folding mechanism in the prokaryotic zinc-finger domain Ros87.

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The correct folding of proteins bearing a zinc finger domain strictly depends on the presence of the metal cofactor, the zinc ion. However, the prokaryotic Cys_2His_2 zinc finger domain (Ros87) found in the Ros protein from *A. tumefaciens* displays a high plasticity (1,2). It is able to fold into a functional three-dimensional structure, capable of binding DNA, even when the endogenous metal ion is replaced by cadmium and still retains a tertiary fold in the presence of nickel and cobalt (3). Nevertheless, the presence of the structural metal ion is found to modulate the folding pathways and the aggregation propensity of this prokaryotic domain (4). For this reason, here we study by means of CD, DSC and NMR the effects of endogenous metal substitution with cobalt, nickel and cadmium on the folding mechanism of Ros87.

References: 1. G. Malgieri, L. Russo, S. Esposito, I. Baglivo, L. Zaccaro, E. M. Pedone, B. Di Blasio, C. Isernia, P. V. Pedone, and R. Fattorusso, PNAS, vol.104, Issue 44, 207 pp.17341-17346
2. G. D'Abrosca, L. Russo, M. Palmieri, I. Baglivo, F. Netti a, I. de Paola, L. Zaccaro, B. Farina, R. Iacovino, P. V. Pedone, C. Isernia, R. Fattorusso, G. Malgieri, J Inorg Biochem, 2016, 161, 91-98.
3. V. Sivo, G. D'Abrosca, L. Russo, R. Iacovino, P. V. Pedone, R. Fattorusso, C. Isernia, and G. Malgieri Inorg Chem. 2019 Jan 22;58(2):1067-1080.
4. G. Malgieri, G. D'Abrosca, L. Pirone, A. Toto, M. Palmieri, L. Russo, M. F. Sciacca, R. Tatè, V. Sivo, I. Baglivo R. Majewska, M. Coletta, P. V. Pedone, C. Isernia, M. De Stefano, S. Gianni, E. M. Pedone, D. Milardi and R. Fattorusso Chem. Sci., 2018, 9, 3290

Insights into the tumor associated protein P150, the largest subunit of the Chromatin Assembly Factor 1

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The Chromatin Assembly Factor 1 is a heterotrimeric complex responsible for the nucleosome assembly during DNA replication and DNA repair. In human, its largest subunit, named P150, was recently considered as a tumor-associated protein due to its overexpression in many malignancies. Structural and functional studies targeting P150 are still limited and only scarce information about this subunit is available. Therefore, the aim of my Ph.D. was to provide insights into the biochemical, structural and functional features of P150. Based on a bioinformatics analysis that assisted the identification of stable domains within the full-length protein, various regions of P150 were cloned, expressed in *E. coli*, purified and subsequently characterized. Results show that the region encompassing the residues 721 to 860 of P150 can bind DNA, no matter its content (AT or GC) and its length (58 or 16 pb). On the contrary, the extended region 575-860 did not bind DNA. This inhibition could be mediated by the conserved acidic domain able to neutralize the basic amino acids involved in the DNA binding activity. Finally, the region covering the residues 880 to 956 of P150 belongs to the family of Intrinsically Disordered Proteins. Despite being natively unfolded, it possesses some elements of polyproline II secondary structure. Taken all together, new structural and functional elements of P150 were provided during my Ph.D., which help to better understand the molecular mechanism of the nucleosome assembly. Further studies are necessary to correlate biochemical and structural features of P150 to its role in cancer.

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

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

One of its monogenic causes is the instability of trinucleotide (GCN) repeats affecting the exon 2 of Aristaless-related homeobox (*ARX*) gene, codifying an interneuron-specific transcription factor with a key role in mammalian corticogenesis and neuronal maturation.

We report on treatment with natural small molecules, already selected in our laboratory in the polyAlanine repeat mouse model, $Arx^{(GCG)7/Y}$, which expresses seven GCG-triplets inserted at residue 330 of the mouse *Arx* gene. This model shows severe spontaneous seizures, a phenotype that recapitulates the chronic epilepsy associated with c.304ins(GCG)7 in *ARX* patients. Using video- monitoring, we have monitored and analysed the behaviour of postnatally treated mice $Arx^{(GCG)7/Y}$ before, during and after administration of purified, botanically derived molecules. Seizure frequency, duration and index were evaluated in each treated-animal with phenotype severity graded on the Racine scale. Safety and tolerability were evaluated based on physical examination and mortality data were collected. Ongoing efforts to define the primary and the secondary endpoints will help to identify innovative therapeutical compounds for malignant EE.

Structure and function of key macromolecules involved in severe humane pathogeneses

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The immune system is a key element in ensuring our survival. My Ph.D. studies focus on studying the modulation of the immune system in response to severe human diseases, using a structural biology approach. On one hand we are trying to modulate the innate immune system in response to several cancers in order to increase the effectiveness of immune therapies against these diseases, and on the other hand we are trying to modulate the adaptive immune system by studying a protein that could be a promising vaccine against tuberculosis.

CD55 is a protein that represses and regulates the innate immune response. It is frequently overexpressed by several cancers in order to possibly avoid the complement-mediated cell lysis. With this in mind, we engineered, developed and produced several molecules that are able to bind CD55 in order to inhibit its action. Using biophysical techniques, we have proved that the developed molecules bind CD55 with high affinity. The next step will be to measure their direct binding affinity to cancer cells. In addition, structural studies are ongoing to understand the mode of binding of these developed molecules to CD55. Connected to the host immune response, my Ph.D. thesis also aims at the structural and functional characterization of a promising vaccine against tuberculosis. This molecule, denoted as Rv2299c, was identified by our collaborator Prof Hwa-Jung Kim at the University of Daejeon, South Korea, in the framework of a collaborative project between Italy and South Korea, of which I am a participant. Although Rv2299c has promising antigen properties in immunization against tuberculosis, nothing is hitherto known on its structure and function. We have characterized it to be a chaperone with ATPase activity and we devised a structural model for it based on the HtpG chaperone of E. coli. Currently, we are trying to experimentally determine its crystal structure and to design better antigens against tuberculosis.

Session 4:
Molecular Cell Biology

Untangling the phosphorylation of tau by understanding the role of novel interplayers: Endocannabinoid system, Orexin, Leptin and LPA

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Neurodegenerative diseases and obesity are causes of worldwide disability and decreased quality of life. A link between both diseases is opening novel research avenues in the search for therapeutic approaches because unfortunately the current available treatments remain ineffective. The metabolic changes caused by obesity are able to modulate the central nervous system by altering synaptic plasticity, which is mainly regulated by the fine balance between the phosphorylated and not phosphorylated tau form.

In this study, we have investigated the changes in synaptic plasticity in certain areas of the brain, starting from the hypothalamus, the most extensively interconnected area accordingly to its inherent functional activities and areas implicated in cognitive function, such as hippocampus and prefrontal cortex, in order to further understand the molecular mechanisms underlying the phosphorylation of tau. GSK3β is a key enzyme in charge of the delicate and important stability of phosphorylation form of tau, protein responsible of modulate synaptic plasticity in these regions.

Several interplayers, like leptin hormone, orexin-A (OX-A) neuropeptide and components of the endocannabinoids (ECs) system are able to interact and modify the activity of GSK3β and different studies reported alterations in obese and neurodegenerative mouse models.

Using molecular, biochemical and morphological studies, we found remarkable changes in the expression of phosphorylated tau form in hypothalamus, hippocampus and prefrontal cortex of leptin knockout ob/ob. This condition was reverted after leptin treatment or using specific inhibitors. We have also observed elevation of 2-arachidonoylglycerol (2-AG) and OX-A content in hypothalamus, hippocampus and prefrontal cortex, confirming that OX-A and 2-AG are interplayers able to increase or decrease, respectively the phosphorylation of tau in opposite way.

In the light of our results, we hypothesize a functional Ox-ALeptinECs interaction as an upstream signaling pathway for the regulation of tau phosphorylation. Unraveling the functional crosstalk between ECs and OX system in the regulation of tau phosphorylation in certain areas of the brain could reveal novel molecular players and pathways that might result in druggable targets.

Mitochondrial dynamics as a new therapeutic target for neurodegenerative diseases

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Mitochondrial homeostasis is vital for cells' survival and presupposes the regulation of their dynamics. Evidently, compromised mitochondrial homeostasis is linked to several neurodegenerative conditions, including Parkinson's Disease, Alzheimer's Disease, and Down Syndrome (DS).

Published data from our lab depict mitochondrial impairment in primary fetal fibroblasts with trisomy 21 compared to the non-trisomic ones. Trisomic cells show prominent mitochondrial dysfunctions, and their morphology is characterized by an imbalanced fusion/fission ratio and cristae fragmentation. Importantly, drugs affecting the mitochondrial biogenesis and rebalancing mitochondrial dynamics could be a possible therapeutic intervention for individuals with DS.

To clearly identify the specific mitochondrial dysfunctions outlined in the trisomic cells, we furthered our investigations focusing on the autophagy process. Most of our acquired data represent the basal autophagy levels, revealing a high variability between the two karyotypes of both LC3B-II levels and LC3-II/LC3-I ratio in western blots. The confocal image analysis of LC3-positive autophagic vacuoles is being further optimized by introducing proper experimental controls.

Much of our current understanding comes from *in vitro* studies in primary fetal fibroblasts and will be extended with experiments in neuronal lineages differentiated from induced pluripotent stem cells (iPSCs). Insights into these mechanisms will better pave the way from bench to bedside.

A combination of exercise with fasting leads to a boost of fatty acid consumption in rats and humans, with beneficial outcomes.

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A novel protocol based on short term fasting in combination with endurance exercise has been tested for beneficial effects in rats and humans. Rats, housed at thermoneutrality (28 C°), exercised for 5 sessions on a treadmill (30 minutes each at 15 m/min, 0° inclination, twice a day for 3 days), showed a strong transcriptional metabolic switch of toward use of lipids as fuel. Thyroid hormone and acylcarnitine levels confirmed an increase of beta oxidation, that we also verified in rat skeletal muscle cells. In addition, increased ketone body levels indicate an increased use of fatty acids. A similar protocol, in which 107 white males were enrolled, comprised a gradual decrease of food supply in 3 days, followed by a 3-day fasting period and a gradual return in 4 days to the initial caloric intake, combined with an endurance exercise. Non-invasive analysis by DXA scan demonstrated a favourable change of body weight composition. Analysis of SNPs related to muscle metabolism failed to show potential genetic predisposition of the participants.

D-Aspartate in the spermatogenesis

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D-aspartate (D-Asp) is an endogenous amino acid presents in vertebrate tissues, with particularly high levels in the testis. Specifically, D-Asp was found in Leydig, Sertoli and germ cells. In vitro and in vivo studies demonstrated that D-Asp up-regulates testosterone synthesis through hypothalamic-pituitary-gonadal axis and/or directly by stimulating in Leydig cells the protein expression of StAR, the key regulatory factor of cholesterol translocation to inner mitochondrial membrane.
To elucidate the functional role of D-Asp in spermatogenesis, we used as models both mutant mice ($Ddo^{-/-}$) with targeted deletion of D-aspartate oxidase (D-AspO) and mutant mice (Ddo OV) which overexpress D-AspO, a peroxisomal flavoprotein which catalyzes the deaminative oxidation of D-Asp. Mice $Ddo^{-/-}$ show selective increase of D-Asp levels in the testis. Interestingly, we found that mice $Ddo^{-/-}$ of two months old showed higher levels of serum/testis testosterone than wild types. The morphological/morphometric analyses of germ epithelium revealed an increase of both spermatogonia mitotic index and seminiferous tubule diameters in mice $Ddo^{-/-}$. In contrast, mice $Ddo^{-/-}$ of six months old did not show significant differences in serum/testis testosterone levels as well as in spermatogenetic activity when compared to wild type mice. The overexpression of D-AspO reduced serum testosterone levels in mice of one month and serum oestradiol levels in mice of three months old. These results strongly suggest an age-dependent role of D-Asp in steroidogenesis and spermatogenesis.

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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The genomic locus miR-99b/miR-let7e/miR-125a-Spaca6 comprises a miRNA cluster located in the first intron of Spaca6 gene. Spaca6 function is still largely unknown, whereas miRNAs belonging to the cluster have been implicated in various physiological pathways, but also in some pathological processes, included oncogenesis where they displayed an oncosuppressive role. Focusing on miR-125a, it has been shown to play a tumor suppressor role in hepatocellular carcinoma by inhibiting cell proliferation, angiogenesis and cell migration through the downregulation of Sirtuin-7, VEGFA, MMP-11, Zbtb7a and c-Raf expression. Intriguingly, a long non-coding RNA (lncRNA) transcribed in the opposite direction has been annotated in human cells, SPACA6-AS, whose first exon sequence is complement to miR-125a and miR-let-7e ones.

The aim of my PhD project can be summarized in two main tasks:

1-Regulation of miRNA cluster expression. miRNA cluster and Spaca6 share the promoter; interestingly, some miR-125a target proteins are in turn involved in the regulation of miR-125a expression, at transcriptional and post-transcriptional level. In particular, the regulatory loop occurring between miR-125a and Zbtb7a, an oncogene for liver cancer, is under investigation.

2- The sequence complementarity between miR-125a/Let-7e and SPACA6-AS suggests the existence of a complex network, wherein miRNAs can regulate the expression of the lncRNA, and the lncRNA could act as a competing endogenous RNA by “sponging” the miRNAs with the subsequent deregulation of their oncogenic targets. The occurrence of the hypothesized loop and its implication in hepatocellular carcinoma is just at the beginning of elucidation.

Novel fluorescent probes for precision labeling in super-resolution microscopy

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The development of novel bioconjugation strategies is becoming a very broad field of research, which has a strong impact on many fields related to life science, such as theranostics and advanced imaging. Currently, a major problem in super resolution imaging systems is related to the precision of labeling the protein target of interest. Indeed, a common problem that plagues every light microscopy technique is related to the use of conventional markers for cellular staining. With antibody probes and classical immunofluorescence protocols, there is always a localization uncertainty of 20-40 nm because the emitters are far from the target of interest. Our research is focused on the development of novel Fab-based staining reagents that are able to break this localization uncertainty. To accomplish this, we have characterized an N-terminal selective reaction to ensure that the fluorescence emitters located there are very close to the epitope. We tested and validated our Fab-based probes using both super resolution imaging and single molecule Forster resonance energy transfer (FRET). Now, our focus is to generate a FRET assay for detecting disease relevant protein-protein interactions, with a particular interest in the Erbb2/Erbb3 heterodimerization process that is aberrant in many cancer types.

3,5 diiodo-L-thyronine (T2) improves the inflammatory response in visceral white adipose tissue (VAT) of rats fed a high fat diet

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Over 30 years of research has demonstrated that T2, an endogenous metabolite of thyroid hormones, exhibits interesting metabolic activities that occur mainly in the liver and skeletal muscle. Considering the role of adipose tissue in metabolic homeostasis, recently we focalized our studies on T2 actions in this tissue. Particularly, it is known that visceral white adipose tissue (VAT) accumulation is associated with inflammation because it is poorly oxygenated and it is exposed to oxidative conditions. In light of the above, the purpose of this study was to test whether T2, in addition to metabolic actions, is able to improve the adipocytes inflammatory response in rats overweight. Three groups of rats were used: i) receiving a standard diet for 14 weeks; ii) receiving a high-fat diet (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 in T2-treated rats, associated to a reduction in adiposity and amelioration in the insulin response, there was an improvement of adipocyte inflammatory response. In addiction, the oxidative damage HFD-induced was reduced after T2 administration. Our studies provided novel findings to explaining the action of this intriguing thyroid hormone metabolite.

In vivo and *in vitro* evaluation of ellagic acid effects on human and animal reproduction

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PhD cycle: 32° ciclo

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Oxidative stress (OS) can impair the human and animal reproduction. Specific antioxidants could protect the organisms from ROS attack and also counteract this condition. My project showed the antigenotoxic potential of the ellagic acid (EA) *in vitro* on sperm and amniotic cells and *in vivo* on Danio rerio embryos. These three different experimental models were exposed to different concentrations of EA and a genotoxic agent, alone and in combination. The *in vitro* data shown a decrease of DFI % (TUNEL) and an increase of GTS% (RAPD-PCR) in sperm and amniotic cells exposed to EA. Contrary to the treatment with the oxidizing agent, that with EA *in vivo* shown no morphological alterations, such as body hypopigmentation, calf sac edema and altered natal movements. Moreover, cytotoxicity and genotoxicity tests (DCF Assay and RAPD-PCR) on embryos, confirmed that the EA exposure did not induce damage to any biological levels, except for the highest concentration tested. According to my results EA could be considered as a powerful antigenotoxic agent, able to protect the earliest stages of ontogenesis from mutagenic substances, moreover it is able to counteract the OS effects also in sperm and amniotic cells, pivotal for the fertilization and development process.

Leprel-1 is involved in angiogenesis process.

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PhD CYCLE: 34° cycle

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In order to identify new genes involved in the angiogenesis process, we performed RNA Sequencing (RNAseq) experiment on primary endothelial cells (HUVEC) stimulated with VEGF-A and we focused our attention on a gene that resulted upregulated, Leprel-1, also known as P3H2 (Prolyl 3-Hydroxylase 2), an enzyme that catalyzes the post-translational modification of collagen 4 proline. Currently, Leprel-1 activity has never been associated to neo-angiogenesis process.
First, we confirmed by qRT-PCR and western blot analyses the data obtained by RNASeq in HUVEC as well as in HMEC primary cells, showing that Leprel-1 modulation is mediated by VEGFR-2. Second, we performed gain- and loss-of-function experiments in HUVEC cells, by the use of expression vector and siRNA transfection, respectively. Proliferation and migration functional assays showed that the change of Leprel-1 expression did not affect HUVECs proliferation whereas cell migration resulted modulated with respect to the expression level of Leprel-1.
Next, we will perform sprouting assay to reinforce the possible involvement of Leprel -1 in angiogenesis. In addition, since Leprel-1 has been also described as tumor suppressor gene, we will generate tumor cell lines stably overexpressing or downmodulating Leprel-1 to perform xenograft tumor experiments.

Delineating cargo classes and their differential requirement of Signalling cascade for exit from the Endoplasmic Reticulum (ER)

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A third of the proteome is hosted by the Endoplasmic Reticulum (ER) for folding, initial post-translational modification, and quality control. The export from the ER is subject to regulation by kinases that rapidly initiate phosphorylation of different downstream targets, at discrete cargo accumulation sites called ER exit sites (ERES). Previous paper from our lab has already outlined a signalling cascade for cargoes such as VSVG and Pro-Collagen which confers the responsibility of their export on Protein Kinase A (PKA) and a few other kinases. The phosphorylation targets of these kinases are hypothesised to be involved in potentiation of folded cargo accumulation at the ERES, enhanced recruitment of proteins in the vicinity of the ERES for packaging and transport of the cargo to the Golgi.

It has been further noted that, PKA does not seem to be the only kinase implicated in cargo export.

Soluble cargoes found localised entirely in the ER have the disadvantage of lack of direct interaction or recruitment of the cytoplasmically residing COPII components which are essential for ER-to-Golgi transport. They are shown to interact with transmembrane cargo adapters found along the membrane of the ER and get transported.
Using model cargoes such as Glycosylphosphotidylinositol (GPI) anchored -GFP and Growth Hormone (GH) -GFP as our models of soluble cargoes, we now show involvement of novel Protein Kinase C (n PKC's) in their exit from ER. Greater spacio-temporal and functional segregation of cargoes and the kinase that are involved their exit, hints at several elaborate auto-regulatory systems at the ERES. These systems not only regulate cargo flux and export but also may have functional downstream implications at organelles that work after the ER along the secretory pathway. For example, the activation of PKA as a consequence of Pro-Collagen export from the ER could trigger phosphorylation of a Golgi protein that might prep the organelle for the incoming protein, arrange the enzymes responsible for its appropriate glycosylation along the cisternae etc.,

The aim of my PhD would be to elucidate the existence of several such an intricate, interconnected and self-regulated circuits at the ERES for different classes of cargoes.

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

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PhD cycle: 32° cycle

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Secreted factors are key mediators of cell-cell communication and altered secreted patterns are linked to the hallmarks of cancer. Secretomes of cancer cells often display altered composition compared to the one of the normal counterpart from which they originated, and these changes can support the extracellular microenvironment modifications that promote tumorigenesis and tumor cell migration/invasion.
Recently, our laboratory identified an emerging class of enzymes, the 1-Acyl-Glycerol-3-Phosphate AcylTransferase enzymes (AGPATs), which localize along the biosynthetic pathways. Four members (LPAATα, LPAATβ, LPAATγ and LPAATδ) are characterized as LysoPhosphatidic Acid AcylTransferase enzymes (LPAATs), able to specifically acetylate the LPA to form phosphatidic acid (PA).
Among them, LPAATδ, localized at the *trans*-Golgi membranes, controls the secretion of several proteins involved in tumor progression and migration/invasion such as the human growth hormone and the membrane type 1- and 9-matrix metalloproteases; moreover, LPAATδ gene is more amplified in prostate cancer (PCa) than in other cancer types. This data support the possible mechanistic link between the increased LPAATδ expression/activation in cancer cells and the altered membrane transport pathways involved in tumorigenesis. Therefore, this study aims to better investigate the role of LPAATδ in the secretion of key cancer factors involved in cells migration/invasion.
We performed a proteomic approach to identify factors differentially secreted in the conditioned medium of PCa *versus* non-cancer cells, by LC-MS/MS. Then, we have used the same approach to identify factors with reduced secretion under LPAATδ depletion followed by statistical analysis (by *Perseus* software). Finally, the proteins identified as secreted (by *SignalP/SecretomeP* software) have been analyzed, in order to select those with increased expression in prostate cancer patients (*cBioPortal for cancer genomics)*. The role of these identified LPAATδ-mediated secreted factors has been further defined in prostate tumor cell migration/invasion.

Characterization of the Cellular targets of the Glycerophosphoinositols

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Glycerophosphoinositols are biologically active metabolites produced from membrane phosphoinositides by phospholipase A_2IValpha. When added exogenously, the glycerophosphoinositols can enter cells and have multiple effects. Previous studies conducted in our lab have unveiled that glycerophosphoinositols impair tumor cell migration through the extracellular matrix by melanoma cells, whereas glycerophosphoinositols act as paracrine factors in a negative feed-back loop that decreases pro-inflammatory response in LPS-treated human monocytes affecting the expression of key pro-inflammatory mediators. With the aim to elucidate the underlying mechanism of action of glycerophosphoinositols, we focused our attention on the ability of glycerophosphoinositol-4-phosphate to induce remodeling of the actin cytoskeleton. Our lab had identified tyrosine phosphatase Shp1 acts as a specific intracellular receptor of this metabolite. In order to characterize and ascertain the residues/domain involved in the regulation of the phosphatase activity and in the binding of glycerophosphoinositols to Shp1, we performed various biophysical methods, NMR spectroscopy, mutagenesis and structural analysis studies, resolving the domain of Shp1 involved in this binding. Since Shp1 was identified by proteomic analysis as a common interactor of both unphosphorylated glycerophosphoinositol (GroPIns) and glycerophosphoinositol-4-phosphate, we further investigated if it is involved also in GroPIns-mediated anti-inflammatory responseThe full definition and validation of the specific protein domain/s involved in the binding of the glycerophosphoinositols will allow the identification of additional cellular receptors and the understanding of the mechanism of action of these compounds in different cell types where they are known to be involved.

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.

Characterization of murine models in imprinting disorders

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

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

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

A mouse line, modelling both disorders, has been previously generated by replacing the endogenous mouse IC1 (mIC1) with the orthologous human IC1 (hIC1) allele. The human allele carries a deletion of 2.2 Kb (hIC1Δ2.2), found associated with some BWS familial cases. Interestingly, the mice show pre/post-natal overgrowth upon maternal transmission of the human knock-in (BWS-like) and pre/post-natal undergrowth upon paternal transmission (SRS-like).

In order to investigate the physio-pathological mechanisms of the two syndromes, we want to deeply characterize the phenotype and the molecular pathways altered in our mouse lines by performing genetic, histological and biochemical-clinical analysis.

Preliminary data show an altered amount of mucopolysaccharides in extracellular matrix of several organs of the mice, according to the parental transmission of the knock-in. This lays the groundwork for investigating a signaling pathway that could explain the differences of the growth phenotype.

Profiling histone modification (H3K4me3) in preadipocytes of individuals with a family history of type 2 diabetes

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Background and aims: First degree relatives (FDR) of individuals with type 2 diabetes (T2D) are characterized by an impaired subcutaneous adipose tissue (SAT) adipogenesis, which is associated with increased risk of developing T2D. Epigenetic factors may regulate this abnormality. We aimed to identify the histone modification profiles in T2D FDR, using an epigenome-wide approach.

Materials and methods: Genome-wide chromatin-immunoprecipitation sequencing approach (ChIP-Seq) for H3K4me3 was applied in SAT Stromal Vascular Fraction cells obtained from lean FDR and control subjects. Bioinformatic analysis of H3K4me3 enrichment was validated by ChIP. Mitochondrial DNA (mtDNA) content was analyzed by qPCR.

Results: We found 2644 differentially enriched regions in FDR and control individuals. Intriguingly, we observed the enrichment in genes belonging to the adipocyte differentiation and mitochondrial biogenesis, including PPARγ, TFAM, COX4I1, and CS. We detected and validated a significant decrease of H3K4me3 on PPARγ, TFAM, COX4I1, and CS promoters of FDR preadipocytes compared to control. There was only a trend in reduction of mtDNA content of FDR preadipocytes. However, mtDNA content was significantly decreased during adipocyte differentiation in FDR. In conclusion, we determined an epigenetic signature in FDR preadipocytes associated with reduction of mitochondrial number during adipogenesis.

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 nephroblastooma, 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). Both ICRs are genomic regions of ~ 2-4 kb in length 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 first performed methylation analysis by pyrosequencing on DNA extracted from blood leukocyte of 13 BWS patients and of 59 WTs. The results confirmed that a subset of BWS shows MMDs and, most importantly, a high percentage of WTs also shows the same epigenetic defects. Interestingly, WT patients with MMDs show a more aggressive phenotype compared with others WTs. Next, we performed genetic mutation analysis by exome-sequencing or CGH arrays to explore the possible cause of MMDs in both diseases. Interestingly, we found that a small subset of BWS shows novel genetic variants in PADI6 gene, a component of the subcortical maternal complex. In contrast, preliminary results suggest that the methylation defects of a subgroup of WTs are mainly caused by chromosome copy number alterations (deletions or duplications).

Neuroimmune mutations leading to dementia: focusing on microglial receptor CD33

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Alzheimer's disease (AD) and Frontotemporal dementia (FTD) are heterogeneous with respect to clinical features and underlying pathologies. They have overlapping clinical symptoms, are multifactorial, associated with many candidate genes and probably involve common molecular pathways that are yet to be understood. Despite extensive research and drug development, there are still no therapies that slow or stop their progression. Disappointing results from recent large-scale clinical trials reflect the lack of methods for early detection and an insufficient understanding of disease mechanisms in humans.

A hallmark of dementia is the activation of inflammatory pathways and mutations in genes related to neuroinflammation, including microglial receptor CD33. These events may be predisposing factors in these diseases and could represent entry points for therapeutic intervention. We clinically characterized patients carrying mutations in CD33, offering us a unique opportunity to identify the CD33 role as disease modifier. We generated microglial cells from human embryonic stem cells (hESCs) in order to create in the next future a patient-derived disease model starting from induced pluripotent stem cells (iPSCs) identifying coding and non-coding transcriptional signatures associated to dementia in order to elucidate the role of new factors in molecular pathways underlying AD, FTD in particular and other neurodegenerative disorders.

Transcriptional and epigenetic deregulation of glycosphingolipid metabolism in Rett syndrome models

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the Methyl-CpG-binding Protein 2 (*MECP2*) gene, encoding an epigenetic modulator of transcription. To date, the pathogenetic mechanism of RTT is still unclear.

Correlations between RTT and glycosphingolipid metabolism alteration are emerging. Glycosphingolipid abnormalities, indeed, were reported in brain of RTT patients and altered levels of gangliosides, glycosphingolipids abundant in neurons, were found in brain of *Mecp2*-null mice. Moreover, mutations in ST3GAL5 gene, encoding a key enzyme for ganglioside biosynthesis, have been found in patients with RTT-like phenotype.

Exploiting cellular and animal RTT-models, we demonstrated that MeCP2 regulates the expression of genes encoding factors involved in both biosynthetic and catabolic pathways of gangliosides, through a direct mechanism. These findings highlighted a novel role of MeCP2 in the control of ganglioside metabolism.

We previously reported that AUTS2, mutated in autism spectrum disorders, promotes neuronal differentiation by regulating glycosphingolipid biosynthetic pathway. Here, we found that MeCP2 directly regulates the expression of AUTS2 in mouse brain, through the binding of its promoter. In the light of these evidences, we speculate that MeCP2 and AUTS2 cooperate for the modulation of ganglioside metabolism and that AUTS2 deregulation, caused by MeCP2 dysfunctions, contributes to RTT pathogenesis.

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 devastating neurodevelopmental disorder, the pathogenetic mechanisms of which are still unknown. RTT phenotype includes motor disabilities, autistic−like features and mental retardation.

Alterations in glycosphingolipid composition in brain of RTT patients have been found. Recently, we highlighted altered glycosphingolipid levels in *Mecp2*-null mice brain. Moreover, MeCP2 directly modulates the expression of glycosphingolipid-related enzymes (GRE).

Glycosphingolipid metabolism in neurons is regulated by AUTS2, an epigenetic factor associated with autism spectrum disorders. We previously demonstrated that MeCP2 directly represses AUTS2 that, in turn, is enriched on *Mecp2* promoter, which suggests a reciprocal regulation.

Against this background, we plan to dissect the role of MeCP2/AUTS2 crosstalk in glycosphingolipid metabolism, by generating cellular and animal models. We will silence MECP2, AUTS2 or both in human neuroblastoma cells (SH-SY5Y) differentiated with retinoic acid and will compare glycosphingolipids and GRE levels. Moreover, to understand the role of AUTS2 dosage in RTT pathogenesis, we will generate a mouse model *Mecp2*$^{-/y}$;*Auts2*$^{+/-}$. We will evaluate the effects

of AUTS2 reduction in a *Mecp2*-null context in terms of both phenotypic score and, from the molecular point of view, of glycosphingolipid and GRE levels.

Identification of genomic variants responsible for pregnancy loss

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PhD cycle: 33cycle

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Pregnancy Loss (PL), the spontaneous demise of a pregnancy before 24 weeks of gestation, occurs in 10-15% of pregnancies. PL is often the result of chromosomal aneuploidies of the gametes but it can also have non random genetic causes like smal-size mutations, both *de-novo* or inherited from parents. Comparative genomic hybridization (CGH) is currently the most accurate method for the genetic analysis of PL, allowing detection of variants of several thousand base pairs. Nevertheless small variants cannot be detected by this technology. My project aims to identify genetic variants likely to cause PL that are not seen by CGH through the analysis of the fetal genome sequence.

Our 119-cases pilot data set complies with the standards of PL described in literature and hence we used it to investigate genetic mutations causing embryonic death. We whole-genome sequenced 18% of the samples in which we do not find obvious aneuploidies by CGH, and prioritized variants according to the severity of their predicted effect. Our preliminary results identify a number of putatively detrimen- tal mutations. Among those, three samples carry two homozygous missense mutations in the AHNAK2 cancer-related gene, coding a cytoplasmic nucleoprotein whose high expression is associated with neg- ative prognosis of several cancers.

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

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Duchenne muscular Dystrophy (DMD) is a X-linked degenerative disorder affecting skeletal muscles and myocardium caused by mutations in the dystrophin gene, mainly deletions and duplications. Point-mutations account for 13% and stop codon mutations are even more unfrequent. A drug treatment for male patients with DMD caused by stop codon gene mutations and still ambulant, has become recently available, based on the clear demonstration of its efficacy in slowing the course of the disease. The drug is able to read through the stop codon. We are treating a still ambulant 27 year-old DMD symptomatic carrier with a stop-codon mutation in exon 53 (c.7792C > T; p.Gln2598Stop). She started the treatment with Ataluren at a dosage of 2,250 mg/die, reporting a prompt subjective improvement in muscle strength. Our results – though preliminary and limited to only one patient – suggest that treatment with Ataluren should be extended to symptomatic DMD female carriers too. After 36 weeks of treatment, our patient refers a greater autonomy in daily life, confirmed by the INQoL test. We aim to follow this patient over time and to recruit other patients.

SQSTM1/p62 Bridges Paget Disease of Bone to Neurological Disorders

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SQSTM1/p62 is scaffold/adaptor protein, and plays a major role as a shuttling factor that targets polyubiquitinated proteins for degradation by either the autophagy or proteasome pathways. Paget Disease of Bone (PDB) is a common metabolic bone disorder, characterized by focal areas of increased bone resorption by osteoclasts, coupled with increased and disorganized bone formation by osteoblast cells. In some patients, PDB have a significant co-existence with ALS. SQSTM1/p62 mutations have been reported in 30-40% of PDB patients and in a small number of patients with ALS and FTD (1-4% with ALS and 2% with FTD). The molecular analysis in patients with both phenotypes was not performed. Recently we have been identified a family with PDB and ALS phenotype. By performing mutation analysis we identified the Y383X mutation in SQSTM1 gene that result in a truncated protein without the UBA domain. UBA domain is important for proteins that bind monoubiquitin or polyubiquitin chains and act as regulators of ubiquitin-mediated processes for proteasomal degradation of proteins. So, our aim is to analyze the truncated mutation Y383X in SQSTM1 gene whether it is responsible to cause both phenotypes or a specific one and study the function of protein in pathological conditions.

Uncovering of ICF syndrome phenotypes in hematopoietic differentiation derivatives from patient- and gene corrected-iPSCs

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PhD cycle: 34°

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The Immunodeficiency, Centromeric instability, Facial anomalies (ICF) syndrome is a very rare autosomal recessive disorder causing immunodeficiency and intellectual disability with different degree of severity. About 60% of patients show mutations in DNA-methyltransferase-3B gene leading to genomic hypomethylation, in particular at (peri)centromeric repeats. It is still unknown why it predominantly affects the immune and neural cells. We plan to elucidate the molecular and phenotypical defects arising during differentiation of ICF patients induced pluripotent stem cells (iPSCs) towards hematopoietic progenitors and lymphoid lineage.

The hematopoietic differentiation of hiPSCs turned out particularly complicated to achieve and, even though different systems were used, there is still high variability regarding the success of the differentiation. We plan to generate CD45+ enriched progenitors from iPSC and to develop suitable approaches to further differentiate the $CD45^{+}$ cells toward the B-cell lineage. We aim at identifying the early-stage molecular and cellular defects caused by DNMT3B dysfunction in disease-relevant cell types. By comparing the differentiation potential of the patient-derived iPSCs with that of isogenic iPSCs-clones obtained following CRISPR/Cas9 correction of the DNMT3B mutations, we will determine whether the molecular and cellular defects exhibited during hematopoietic differentiation may be rescued and obtain mechanistic insights into the underlying molecular events.

A new strategy for phase specific mutation detection

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Pathogenetic structural variants are common in human genomes and reliable identification of these remains challenging. Many small structural rearrangements, as well as point mutations in the highly repeated regions of the genome or in genes with similar pseudogenes, are still lost in molecular diagnosis. The recently developed linked-read sequencing technology from 10X Genomics combines a new barcoding strategy with Illumina NGS. 10x Genomics technology is based on the partitioning of samples and reagents into droplets that contain uniquely barcoded beads called GEMs (Gel Bead-In EMulsions). The fragments of genomic DNA, that derive from a given long fragment, are divided into separate micro-reactions. However, all reads from a respective GEM contain identical barcodes and can later be assigned to groups originating from the same DNA molecule. The assay requires low input of high molecular weight DNA and produces "linked read" information which can be used to assemble genomes, assess structural variants and reconstruct large scale haplotype. During my PhD project, I will apply this technology to identify variations in very similar genes, such as SMN1 and SMN2, or in repeated exons, such as titin and nebulin genes, or in genes with similar pseudogenes, such as PKD1 and PKD2.