Università degli Studi della Campania *Luigi Vanvitelli*

PhD Days 2021
PhD Programme in Molecular Life Science

Cancer biology, Immunology, Microbiology, Drug design

Alessandro Verde
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Tutor: Giovanna Del Pozzo

Annachiara Sarnella
Bone Marrow Mesenchymal Stem Cells contribute to cisplatin resistance through CA IX overexpression in Triple Negative Breast Cancer
Tutor: Prof Antonella Zannetti

Gene Regulation and Computational Biology

Valeria Policastro
Multilayer Network Omic Integration
Tutor: Prof. Annamaria Carissimo, Prof Claudia Angelini

Francesco Cecere
Investigating the causes of DNA methylation disturbances in the Beckwith-Wiedemann syndrome
Tutor: Prof. Andrea Riccio, Prof. Flavia Cerrato

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

Karla Alejandra Ruiz Ceja
Meta-analysis of human retinal transcriptome data: a powerful tool to gain insight into the organization of inherited retinal disease genes and to identify putative interactors
Tutor: Prof. Sandro Banfi

Structure and Functions of Biomolecules

Mariangela Valletta
Investigating molecular determinants of cancer by cutting-edges high resolution mass spectrometry technologies
Tutor: Prof. Angela Chambery

Angela Clemente
Characterization of quinoin, type 1 ribosome inactivating protein from Chenopodium quinoa Willd seeds
Tutor: Prof. Antimo Di Maro

Mariateresa Allocca
Small molecules for the treatment of PMM2- CDG
Tutor: Prof. Giuseppina Andreotti

Veronica Russo
Prokaryotic and Eukaryotic zinc-finger proteins
Tutor: Prof. Paolo Vincenzo Pedone

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

Vikram Pratap Singh
Innovative mass spectrometry-based strategies for unraveling the Macrophage migration inhibition factor (MIF) interactome
Tutor: Prof. Angela Chambery

Mario di Gennaro
Hyaluronic acid and its derivatives based multifunctional nanostructured devices in cancer therapy and regenerative medicine
Tutor: Prof. Assunta Borzacchiello

Valentina Verdoliva
Development of biocompatible hyluronan-based materials as drug-carriers and implant systems for tissue engineering
Tutor: Stefania De Luca

Rinaldo Grazioso
Effects of the change in buffer conditions and of the substitution of the structural ion on the folding mechanism of Ros87
Tutor: Prof. Carla Isernia

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

Diana Santos
Dissecting the interaction of DDX11 with Timeless, a fork-protection complex subunit
Tutor: Dr. Francesca M. Pisani

Maria della Valle
Impact of nano-plastics on the structure, dynamics and function of (bio)macromolecules and biological systems
Tutor: Prof Roberto Fattorusso

Mohammad Mahtab
Elucidating the role of DDX11, the Warsaw breakage syndrome DNA helicase, at the DNA replication fork
Tutor: Prof Francesca M. Pisani

Alessandra Del Bene
Ultrasonic-Assisted Synthesis of Peptide Nucleic Acids
Tutor: Prof. Anna Messere

Marica Sassano
Nanoplastics impact on 3 different human gut commensals
Tutor: Prof. Gaetano Malgieri

Francesca Guzzo
NMR spectroscopic identification of secondary metabolites extracted from natural sources and evaluation of their potential antibacterial effect on Staphylococcus aureus
Tutor: Brigida D'Abrosca

Joyce Rodriguez
Role of Allelopathy in the Success of Selected Invasive Plant Species in the Mediterranean Basin and Possible Applications
Tutor: Monica Scognamiglio

Clementina Acconcia
Development and optimization of natural-abundance NMR techniques for studying structure and dynamics of proteins in solution and peptide-ligand interactions on the surface of living cells
Tutor: Prof. Luigi Russo

Manil Kanade
Structural and biochemical analysis of FeS DNA helicases
Tutor: Dr. Silvia Onesti

Federica La Rocca
Effects of Glutamate on C. elegans Spinal Muscular Atrophy models
Tutor: Prof Elia Di Schiavi

Haritha Asha
Exciton and charge separation: computational models
Tutor: Roberto Improta

Molecular Cell Biology

Seyedehnegar Parizadeh
Links between the autoregulation of apical cargo export from the TGN and the control of TNBC cell growth
Tutor: Dr. Alberto Luini

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

Armando Di Palo
The long non-coding RNA SPACA6-AS1, miR-125a and its mRNA targets establish a novel ceRNA regulatory network in hepatocarcinoma cells
Tutor: Prof. Nicoletta Potenza

Arianna Cuomo
Direct effect of ketone bodies and thyroid hormone on BDNF maturation in muscle cells
Tutor: Prof. Pieter De Lange

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

Paola Pignata
Prolyl 3-hydroxylase 2 is a molecular player of angiogenesis
Tutor: Prof. Sandro De Falco

Roberta Simiele
Effect of ketone bodies and BDNF on thyroid hormone action in cerebral cortex of rats subjected to exercise and fasting
Tutor: Prof. Pieter De Lange

Giuseppe Petito
Short-Term Fructose Feeding affects insulin signaling and lipid metabolism by modulating microRNAs expression differently in young and adult rats
Tutor: Prof. Antonia Lanni

Martina Garofalo
Role of D-aspartate oxidase on brain development and in neurodevelopmental disorders
Tutor: Prof. Alessandro Usiello

Marta Mallardo
Adiponectin protects against the neuronal damage induced by cerebrospinal fluid from multiple sclerosis via reducing oxidative stress and modulating INFγ expression
Tutor: Prof. Aurora Daniele

Alessia Casamassa
Patient specific cell lines as a model for Parkinson's disease associated to the GBA variant E326k
Tutor: Prof. Massimo Carella; Prof. Jessica Rosati

Ana Sofia Cabaço Boavida
Exploring the role of the FANCJ DNA helicase in counteracting replication stress
Tutor: Prof Francesca Pisani

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

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

Human genetics

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

Martina Di Guida
AAV-mediated microRNAs modulation as gene-independent strategy in inherited retinal dystrophies
Tutor: Sandro Banfi, Sabrina Carrella

Barbara Morone
Hematopoietic differentiation of induced pluripotent stem cells (iPSCs) derived from patient with the Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome into HPCs expressing hematopoietic markers (CD34, CD43, CD45)
Tutor: Dr. Maria R. Matarazzo

Maria Elena Onore
Unsolved rare cases: towards new diagnostic strategies
Tutor: Dr. Vincenzo Nigro

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

Sharon Russo
Zfp687^{D537R} -knock in mouse model exhibits features of Paget's disease of bone and an altered bone marrow composition
Tutor: Dr. Fernando Gianfrancesco

Abu Saadat
Genome-wide studies for the molecular characterization of isolated Wilms tumor
Tutor: Prof Andrea Riccio

Romina D'Alterio
The role of miR-181 in Parkinson Disease
Tutor: Prof. Brunella Franco, Dr A. Indrieri

Pasqualina Cennamo
Genetics of oxidative stress in a population-based study
Tutor: Prof. Marina Ciullo

Giorgio Fortunato
Development of innovative diagnostic protocols for the prediction, progression and monitoring of Parkinson's disease
Tutor: Dr. Teresa Esposito

Silvia Buonaiuto
A pipeline for prioritization of putatively damaging genetic variants in cases of oocytes/embryo developmental arrest.
Tutor: Prof. Vincenza Colonna

Paola D' Ambrosio
A pipeline for prioritization of putatively damaging genetic variants in cases of oocytes/embryo developmental arrest.
Tutor: Prof. Vincenzo Nigro

Session 1:

Cancer biology, Immunology, Microbiology, Drug design

Hybrid lipid/polymer nanoparticles to assist nucleic acid therapeutic lung delivery in cystic fibrosis

PhD student: **Gemma Conte**

Tutor: **Ivana D'Angelo** (ivana.dangelo@unicampania.it)

PhD cycle: **34° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Inhalable nucleic acid therapy has a unique potential for treatment of severe lung diseases, such as cystic fibrosis (CF). Nevertheless, a drug delivery system tackling lung barriers is mandatory to enhance the drug efficacy. To this aim, inhalable hybrid core-shell nanoparticles (hNPs) made up of a combination of lipids and polymers have been developed. hNPs were composed by a poly(lactic-co-glycolic acid) (PLGA) and endogenous phospholipid (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine-DPPC- or 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol) -DSPE-PEG-). In a first approach, hNPs were developed for the delivery of a *siRNA* pool against nuclear factor-κB (NF-κB), critical signal in inflammatory response in CF. Particular attention was focused on the ability of hNPs to assist the diffusion across the mucus layer, employing artificial mucus (AM) and sputa derived from CF patients. The diffusion profiles of hNPs were highly influenced by the composition and viscosity of the sputum samples, the presence of PEG increased the permeation in poorly colonized CF sputa, while a lower permeation was observed in more complex samples. *In vitro* uptake studies on mucus-covered Calu-3 monolayers confirmed that DPPC hNPs were more efficiently internalized than their PEGylated counterpart. In the light of these remarkable results, DPPC hNPs were chosen for the encapsulation of a peptide nucleic acid (PNA), recently considered for its ability to modulate the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The *in vitro* release kinetics of hNPs and their ability in PNA delivery across the human epithelial airway barrier, were investigated. Confocal analyses demonstrated the ability of hNPs to overcome the mucus barrier and release the PNA within the cytoplasm. All the obtained results highlight the great potential of hNPs as carriers for nucleic acid therapeutic lung delivery in cystic fibrosis.

Design and Synthesis of FMRP mimetic cyclopeptides as G-quadruplex stabilizers

PhD student: **Antonia D'Aniello**

Tutor: **Salvatore Di Maro** (salvatore.dimaro@unicampania.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento Di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche, Università Della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta.

Fragile X Syndrome (FXS) represents the most common form of hereditary intellectual disability. The FXS onset is correlated with the loss of expression of the Fragile X Mental Retardation Protein (FMRP). The latter is able to binds specific mRNAs (mainly those encoding proteins involved in the synaptic plasticity and the neuronal development), via two binding sites, namely the 3-K (KH0-KH1-KH2) and Arg-Gly-Gly (RGG) domains. The RGG domain interacts with specific mRNA by binding G-quadruplex structures within the nucleic acid sequence. Recently, Vasilyev et al. explained at molecular level that the binding of the RGG domain with the G-quadruplex sequence strictly depends on the presence of a type I β-turn in the peptide sequence. Starting from these studies, we hypothesized that the FMRP functionalities could be resembled by mimicking the RGG domain. To this end, during my first year of PhD, I synthesized a small library of cyclopeptides by introducing at the N- and C-termini of RGG core sequence two flanking amino acid mimetics, which were exploited to generate the desired macrocyclization. A total of 8 peptides were synthesized, purified, characterized and are actually under evaluation for their activity and cytotoxicity.

The PD-L1 immune profile in lung cancer and COPD

PhD student: **Renata Esposito** (renata.esposito@unicampania.it)

Tutor: **Bruno D'Agostin**o (bruno.d'agostino@unicampania.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

PD-1/PD-L1 are immune checkpoints that play a key role in immune homeostasis. The up-regulation of PD-L1 on tumor cells and tumor associated macrophages inhibits an effective antitumor immune response. Notably, the phenotypic changes of macrophages have been implicated not only in tumor microenvironment but also in inflammatory diseases like chronic obstructive pulmonary disease (COPD). Moreover, alveolar macrophages (AMs) coordinate inflammation in COPD, promoting the small airway fibrosis and emphysema. While the role of PD-1/PD-L1 pathway is known in lung cancer, its biology in the context of COPD is still unclear. COPD and lung cancer share cigarette smoke as a major risk factor and often occur as comorbidities. Here, we performed a prospective observational study on 190 age- and sex-matched subjects with a suspected diagnosis of lung cancer undergoing routine bronchoscopy and bronchoalveolar lavage (BAL). Out of 190, only 83 patients fulfilled the inclusion criteria. We divided the patients in healthy never smokers (n = 16), smoker controls (n=17), patients with lung cancer (n = 34) and patients with COPD (n=16). Then, the latter were divided according to the GOLD guidelines as GOLD 1-2 (n=9) and GOLD 3-4 (n=7). So, we'll investigate the PD-L1 immune profile on AMs taken by BAL collected from all patients using immunofluorescence; we'll also assess the effect of cigarette smoke on PD-L1 mRNA levels in vitro model of macrophages (THP-1) and in BAL-derived AMs.

Isolation, screening and genomic characterization of marine bacterial isolates involving in pollutant remediation and biomedical applications

PhD student: **Janardhan Ausuri** (janardhan.ausuri@unicampania.it)

Tutor: **Donatella de Pascale** (donatella.depascale@szn.it)

PhD cycle: **34° cycle**

Affiliation: Institute of Biochemistry and Cell Biology (IBBC), National Research Council, Via Pietro Castellino 111, 80131 Naples, Italy & DiSTABiF, University of Luigi Vanvitelli, Caserta.

Extreme marine environments are potential sources of novel microbial isolations with dynamic metabolic activity. *Dietzia psychralcaliphila* J1ID and *Rhodococcus* sp. I2R were isolated from sediments originated from Deception Island, Antarctica and Southern Tyrrhenian Sea respectively grown over phenanthrene. *D. psychralcaliphila* J1ID assessed for its emulsifying activity. In liquid MSM media, *D. psychralcaliphila* J1ID showed 84.66% degradation of phenanthrene examined with HPLC-PDA. The identification of metabolites by GC-MS combined with its whole genome analysis provided the pathway involved in the degradation process. The strain possesses the genetic compartments for a wide range of toxic aromatic compounds, which includes the *benABCD* and *catABC* clusters. Insights into assessing the depletion of phenanthrene throughout the incubation process and the genetic components involved have been analysed. The same bacterium was tested for its heavy metal resistance towards Arsenic, Copper, Zinc. The maximum tolerance concentration assay was done. To analyse the percentage efficiency of precipitation of heavy metals by the strain, ICP-MS was performed. The isolate displayed high heavy metal resistance efficacy in order of Zinc > Copper > Arsenic. In addition, I have been involved in another research project in which the whole genome of a marine isolate *Rhodococcus* sp. I2R was sequenced and analysed by antiSMASH for the identification of Biosynthetic Gene Clusters. The strain was cultivated in 22 different growth media and the generated extracts were subjected to metabolomic analysis and functional screening (antiviral, antiproliferative, biosurfactant). Interestingly, only one growth condition induced the production of unique compounds which were partially purified and structurally characterized by liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS). The active fraction showed a potent antiviral effect against enveloped viruses such as Herpes virus and Human Coronaviruses and mild antiproliferative activity.

Induction of anti-tumor response by filamentous bacteriophage targeting

PhD student: **Roberta Manco** (roberta.manco@unicampania.it)

Tutor: **Piergiuseppe De Berardinis** (p.deberardinis@ibp.cnr.it)

PhD cycle: **35° cycle**

Affiliation: Institute of Biochemistry and Cell Biology (CNR) – Via Pietro Castellino, 111, 80131 Napoli

Here we propose the immunotherapeutic use of filamentous bacteriophages. Indeed, the phage particles, which based on their size can be considered as nature-made nanoparticles, have capability to enter blood vessels, and display antigenic peptides at high-density on their surface. The exposed peptides can be processed and presented after bacteriophage internalization by antigen-presenting cells (APCs) and triggering strong immune responses. Previous studies have shown the ability of bacteriophages, delivering tumor-associated antigens (TAAs) to murine DCs via specific receptor, to improve antigen-specific T-cell responses without exogenous adjuvant. Moreover, bacteriophages can be conjugated to the immunostimulatory lipid alpha-GalactosylCeramide (α-GalCer), a glycolipid known to exert anti-tumor cell cytotoxicity. The delivery of stimulatory lipids and antigenic peptides derived from TAAs may thus represent a novel strategy to potentiate anti-tumor immune responses. In order to improve anti-tumor immunity mediated by bacteriophage delivering TAA and α-GalCer, we target the bacteriophage directly to tumor cells.

We constructed and produced bacteriophages expressing TAAs and/or α-GalCer, together with the antibody fragment anti-human PD-L1. These phage particles specifically recognized PD-L1-expressing tumor cells. B16-OVA melanoma cell line was engineered for the expression of the human PD-L1 molecule by lentiviral transduction. The anti-tumor immune response induced by the recombinant bacteriophages was analyzed in vivo in B16-OVA-hPD-L1 melanoma-engrafted mouse model of vaccination.

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

PhD student: **Mario Campanile**[1,2] (mario.campanile93@gmail.com)

Tutors: **Prof. Giuseppe Pignataro**[1], **Prof. Luca Colucci D'Amato**[2](gpignata@unina.it; luca.colucci@unicampania.it)

PhD cycle: **34° cycle**

Affiliations: 1 Division of Pharmacology, Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy; 2 Laboratory of Cellular and Molecular Neuropathology, Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania, "Luigi Vanvitelli", Caserta, Italy.

Introduction and aims: The limited therapeutic options for ischemic stroke treatment render necessary the identification of new strategies. Therefore, the aim of the present study was to evaluate the protective effect of Ruta graveolens water extract (RGWE) in vitro and in an *in vivo* experimental model of brain ischemia.

Methods: RGWE effects on cell viability were investigated in primary neuronal and glial cultures, whereas the effect on ischemic damage and neurological function was evaluated in adult rats subjected to transient occlusion of Middle Cerebral Artery (tMCAO), receiving intraperitoneal injections of RGWE 100 and 300 minutes after ischemia. In addition, astroglial activation was measured as GFAP expression by immunofluorescence and confocal microscopy analysis.

Results: RGWE preserved cell viability in primary neuronal and glial cultures and may modulate microglial activation. Treatment with 10 mg/kg RGWE ameliorates the ischemic damage and improves neurological performances. Interestingly, GFAP signal, known to correlate to brain inflammation, was significantly reduced in ipsilateral cortical and striatal area in ischemic RGWE-treated rats.

Conclusions: RGWE has a neuroprotective effect in a cerebral ischemia, this effect is paralleled by a prevention of astroglial activation. Collectively our findings support the idea that natural compounds may represent potential therapeutic strategies against ischemic stroke.

Evaluation of risk of Celiac Disease related to the expression of HLA DQ2.5 genes

PhD student: **Mariavittoria Laezza** (mariavittorialaezza@libero.it)
Tutor: **Giovanna Del Pozzo** (giovanna.delpozzo@igb.cnr.it)
PhD cycle: **35° cycle**
Affiliation: Istituto di Genetica e Biofisica (IGB) "A. Buzzati-Traverso", CNR Napoli

Celiac disease (CD) is an autoimmune disorder that occurs in genetically predisposed individuals following the ingestion of gluten. Genetic susceptibility to CD is conferred by HLA class II DQ2/DQ8 molecules, exposed on the surface of immunocompetent cells called APCs (Antigen Presenting Cells) and presenting gluten peptides. APCs activate antigen-specific $CD4^+$ T lymphocytes that, through the production of proinflammatory cytokines, lead to damage of the intestinal mucosa.Recent findings showed that the strength of inflammatory response is mainly determined, on B-LCLs, by the great expression of HLA class II risk genes, HLA-DQA1*05 and HLA-DQB1*02 alleles, either in *cis* and in *trans* configurations, encoding DQ2.5 molecules.During my first year of the project, I compared the expression of risk alleles in PBMCs from patients with acute disease respect to patients in gluten-free diet, carrying different HLA heterozygous genotypes. The mRNA quantification, performed by qRT-PCR with allele specific primer pairs, confirmed the great expression of DQA1*05 and DQB1*02 alleles respect to non-associated ones, but no differences in their expression among the group of celiac patients at diagnosis and celiac patients in remission. Therefore, these results showed that there is a common regulatory mechanism, at the haplotype level, determining the comparable expression of the risk alleles in the two groups of patients.

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

PhD student: **Nicoletta Campolattano** (nicoletta.campolattano@unicampania.it)

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

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Tuberculosis still represents a worldwide health concern, worsened by the appearance of *Mycobacterium tuberculosis* (*Mtb*) multidrug-resistant strains. Here we explore the role of efflux pumps in drug tolerance mechanisms using the model organism *Mycobacterium smegmatis* mc^2155. Our work is based on previous studies describing the function of the MSMEG_3765 protein, a Tet-R like repressor that regulates the *MSMEG_3762/63/65* operon and *Rv1687c/86c/85c* in *Mtb* (Perrone *et al*; 2017). The *MSMEG_3762* and *MSMEG_3763* genes are known to encode for components of an ABC transporter in *M. smegmatis*, as well as their orthologues in *M. tuberculosis.* To characterize the efflux system, the deletion mutant *M. smegmatis* (Δ*MSMEG_3763*) was generated, and comparative analyses with the wt strain, performed in presence of different antimicrobials, suggest the ability of the MSEMG-3762/63 to bind and extrude rifampicin and ciprofloxacin (respectively first- and second-line anti-TB drugs); these findings were also supported by bioinformatics analyses and molecular docking simulations (De Siena et *al;* 2019). To further investigate the effects of the identified putative ligands on the de-repression mechanism underlying the regulatory system of the *MESMEG_3762* operon, electrophoretic mobility shift assays (EMSA) and RT-q PCR analyses were carried out. Moreover, the functional role of the efflux pump in membrane potential alteration was evaluated by cytofluorimetric analyses. Since the *MESMEG_3762* operon is up-regulated in acid-nitrosative stress (Cossu *et al*; 2013), which is a condition mimicking the macrophage environment, its expression after infection of macrophages with *M. smegmatis* cells is under study.

Metabolic rewiring in thyroid carcinomas induced by BRAF gene mutations

PhD student: **Caterina Perfetto** (cate.perfetto@gmail.com)
Tutor: **Dr. Valerio Costa** (valerio.costa@igb.cnr,it)
PhD cycle: **36°cycle**
Affiliation: Institute of Genetics and Biophysics "Adriano Buzzati-Traverso", National Research Council, Naples, Italy

Metabolic reprogramming represents a cancer hallmark conferring proliferative advantage and adaptation to pharmacological therapy. Differential metabolic rewiring can be driven by distinct oncogenic alterations. In papillary thyroid carcinoma (PTC), the mutually exclusive *BRAF* and *RAS* mutations drive a differential MAPK pathway activation, cell proliferation and invasiveness. Despite the detailed molecular characterization of PTCs, the subtype-specific metabolic reprogramming has not been yet explored. One of the aims of the project, accomplished during the 1st PhD training year, was to identify mutation-specific expression signatures of metabolic genes in *RAS*- and *BRAF*-like PTCs. Taking advantage of public whole-exome/transcriptome data deposited in The Cancer Genome Atlas (TCGA) web portal, I contributed to identify a differential pattern of expression between PTC subtypes for genes responsible of energy metabolism. The analysis revealed that the over-expression of genes accounting for glucose uptake, glycolysis and lactate production/excretion - paralleled by the down-regulation of OXPHOS and TCA cycle's genes - is a *BRAF*-like tumors' hallmark. Additionally, methylation analysis of CpG islands indicated that the deregulation of these genes does not associate with substantial methylation changes between PTC subtypes. In line with this, the 2nd year of my PhD will be focused on exploring other regulatory mechanisms, such as transcription factors, possibly accounting for the specific gene-pattern expression identified as *BRAF*-like tumors' hallmark.

SERS-sensing, imaging and nanotoxicology of bacterial endotoxin on gold nanoparticles

PhD student: **Alessandro Verde** (alessandro.verde@ieos.cnr.it, alessandro.verde@unicampania.it)

Tutor: **Anna Chiara De Luca** (annachiara.deluca@ieos.cnr.it); **Paola Italiani** (email: paola.italiani@ibbc.cnr.it)

PhD cycle: **34° cycle**

Affiliation: Istituto per l'endocrinologia e l'oncologia "Gaetano Salvatore" (IEOS), Consiglio Nazionale delle Ricerche (CNR), Via Sergio Pansini 5, 80131, Napoli

Istituto di Biochimica e biologia cellulare (IBBC), Consiglio Nazionale delle Ricerche (CNR), Via Pietro Castellino 111, 80131, Napoli

Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

In recent years, gold nanoparticles (AuNPs) have gained increasing attentions for the development of new nanodevices for a diverse array of biomedical applications. Unfortunately, in some cases, they induce toxic effects on biological systems. Among these, the activation of the innate immune system (inflammatory response) is considered a central issue for assessing health risks. Although the origin of nanotoxicity is not well known, the cause could be ascribed with the presence of bacterial endotoxin on nanoparticles surface. Bacterial endotoxin, also known as Lipopolysaccharide (LPS), is the main component of gram-negative bacteria cell walls and is considered one of the major contaminants in the environment. LPS presence can be detected with different assays but their use on AuNPs can be complicated because NPs themselves could interfere with assay components or with the final readout, leading to unreliable results. For these reasons, the need to develop alternative methods for LPS detection on NP surface is of main importance. Surface enhanced Raman spectroscopy (SERS) represents an excellent tool for molecular detection, because it can amplify the Raman signals of a given molecule adsorbed onto metallic nanosurfaces, as gold NPs, up to 6-7 orders of magnitude. Therefore, due to the Raman fingerprint, SERS-based sensing allows to identify and quantify molecules with excellent sensitivity and reproducibility in different environments, thereby enabling the use of the SERS technique for numerous biomedical and biosensing applications. In this study, we demonstrate the use of SERS approach for quantitative detection of low LPS amount on AuNPs. Moreover, NPs inflammatory effect was assessed in relation of pro and anti-inflammatory cytokine production by *human primary cell-based assay*.

Biotechnological application of *Pseudomonas gessardii* M15 rhamnolipids

PhD student: **Carmine Buonocore**
(carmine.buonocore@unicampania.it;carmine.buonocore@szn.it)

Tutor: **Donatella de Pascale** (donatella.depascale@szn.it)

PhD cycle: **35° cycle**

Affiliation: Department of Marine Biotechnology - Stazione Zoologica Anton Dohrn, Villa Comunale 80121 Naples, Italy

Coronavirus pandemic and multidrug-resistant bacteria highlighted the need for new strategies with non-specific mechanism of action. Rhamnolipids are microbial biosurfactants displaying a wide range of bioactivities, such as antibacterial, antifungal, and antibiofilm, among others. Being of microbial origin, they are environmental-friendly, biodegradable, and less toxic than synthetic surfactants. Previously, we characterized and investigated the antibacterial activity of a rhamnolipids' mixture produced by *Pseudomonas gessardii* M15. Now, we explored the antiviral activity against enveloped viruses belonging to *Coronaviridae* and *Herpersviridae* families, paying attention to the rhamnolipids' mechanism of action. Moreover, we evaluated the biotechnological applications of the mixture, testing its ability to inactivate viruses on treated surfaces and inhibit *Staphylococcus aureus* growth on treated wound-dressing, helping the healing process. Data show a complete inactivation of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), human coronavirus strain 229E (HCoV-229E), and Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), at different concentrations, achieving good results also against human coronavirus strain OC43 (HCoV-OC43). On the other hand, the main wound-healing results were obtained towards *S. aureus* with coated cotton swabs. These outcomes show the potential of rhamnolipids as additives in both pharmaceutical and cosmetics formulations to counteract enveloped viruses and recalcitrant wound infections.

Structural and functional characterisation of novel vaccine antigens

PhD student: **Eliza Kramarska** (eliza.kramarska@gmail.com; eliza.kramarska@unicampania.it)
Tutor: **Rita Berisio** (email: rita.berisio@cnr.it)
PhD cycle: **36° cycle**
Affiliation: Istituto di Biostrutture e Bioimmagini, Consiglio Nazionale delle Ricerche, Via Mezzocannone 16, 80134, Napoli

Structure-based antigen design approach is a valid strategy for next-generation vaccine development. To this aim, the structural definition of the overall atomic-resolution structure of an antigen of its epitope regions is the driving force in the production of engineered antigens with improved immunological properties and provides biophysical tool that facilitate vaccine design and production. *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter sp.* build the ESKAPE group of pathogens, that possesses an enormous capacity to develop a high level of resistance to antimicrobials and cause severe infections, whose outcome can be fatal, especially for immunocompromised hospitalized patients. We are facing a growing problem, that desperately needs a solution. One of our responses to this problem can be creating vaccines that allow to produce long-lasting immune memory and will protect from developing dangerous diseases. Moreover, vaccines can diminish the usage of antibiotics, which is one of the major steps to combat the war against the spread of antibiotic resistance in bacteria. My PhD project, in the framework of the Marie Sklodowska-Curie action BactiVax – Anti-Bacterial Innovative Vaccines, aims at exploiting structural biology methods to develop novel vaccine antigens against ESKAPE pathogens.

Human Carbonic Anhydrase Inhibitors and Activators: A Computational Medicinal Chemistry perspective

PhD student: **Rahul Ravichandran** (rahul.ravichandran@unicampania.it)

Tutor: **Sandro Cosconati** (sandro.cosconati@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Today, molecular modeling techniques play a major role at the various stages of drug discovery pipeline. In this drug discovery context, molecular docking is a computational methodology that is used to provide a snapshot of various types of molecular interactions and binding modes that could be seen between the protein and the ligand. Our current focus is on employing advanced docking protocols like AutoDockZn forcefield to rationalize SARs attained on inhibitory agents against various hCAs as well as the activating agents in favor of hCA isoforms. In our first study, a set of theoretical investigations were carried out on the evaluation of the biological inhibitory activity of a set of new bicyclic tetrahydroindazoles featuring a secondary sulfonamide against hCA I, II, IV, and IX isoforms. These in silico studies helped to dissect the new molecules into the single portions influencing the zinc chelation properties and the selectivity profile thereby offering a new platform for the discovery of new isotype selective CA inhibitors. Based on our first study, we further improved the selectivity and activity by decorating the compounds with N-phenyl secondary sulphonamides featuring the bicyclic tetrahydroquinazole scaffold towards tumor-related hCA IX isoform. Molecular modeling studies were implemented to rationalize the SAR in terms of activity and selectivity. Apart from the inhibitors, carbonic anhydrase activators (CAAs) also represent a novel approach for the treatment of Alzheimer's disease, dementia, aging etc. In this activation study, we aimed at developing novel CAAs having various series of indole-based derivatives with promising selectivity profiles towards the brain-associated cytosolic isoform hCA VII. Molecular modeling suggested a theoretical model of the complex between hCA VII and the new activators and provide a possible explanation for their modulating as well as selectivity properties.

Future prospects: Exploring various advanced docking strategies to provide novel hints from computational medicinal chemistry perspective.

Bone Marrow Mesenchymal Stem Cells contribute to cisplatin resistance through CA IX overexpression in Triple Negative Breast Cancer

PhD student: **Annachiara Sarnella** (achiara.sarnella@gmail.com)

Tutor: **Antonella Zannetti** (antonella.zannetti@cnr.it)

PhD cycle: **35° cycle**

Affiliation: Istituto di Biostrutture e Bioimmagini (IBB-CNR). Via Tommaso de Amicis 95, Napoli.

Triple negative breast cancer (TNBC) is a heterogeneous disease, and even though it occurs in only 15-20% of all patients with breast cancer, it is characterized by an extremely high rate of mortality due to metastatic and drug-resistance recurrent disease. Chemotherapy is the primary established systemic treatment for TNBC patients in both the early and advanced-stages. Many evidences highlight the crucial role played by stromal cells in the hypoxic tumor microenvironment in promoting tumor growth, metastasis and chemoresistance. We aimed to investigate tumor-educated mesenchymal stem cells (TE-MSCs) function in supporting TNBC aggressive behavior through hypoxic-induced protein carbonic anhydrase IX (CA IX). CA IX is an enzyme involved in pH regulation, transcriptionally regulated by HIF-1α in malignant progression of solid tumors and it is overexpress in hypoxic conditions (1% O_2). We observed that TNBC cells grown in 1% O_2 in presence of conditioned medium derived from bone marrow mesenchymal stem cells (CM-BM-MSCs) showed an increase of CA IX levels respect to cell grown in hypoxic and normoxic conditions (21% O_2). TNBC cells treated with CM-BM-MSCs in 1% O_2 enhanced the ability to form spheroids with stemness features and resulted more resistance to cisplatin. The addition of CA IX inhibitor, SLC-0111, sensitized TNBC cells to chemotherapy and reduced their ability to invade extracellular matrix.

Session 2:

Gene Regulation and Computational Biology

The role of Padi6 in genomic imprinting. A new mutant mouse model

PhD student: **Carlo Giaccari** (carlo.giaccari@unicampania.it)

Tutor: **Andrea Riccio** (andrea.riccio@unicampania.it)

PhD cycle: **34° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

In mammals, a subset of genes, with a fundamental role in the embryo-development, is characterized by monoallelic and parent-of-origin-dependent expression. This phenomenon is known as genomic imprinting. It is regulated by epigenetic mechanisms as DNA methylation and histone modifications. A number of diseases characterized by abnormalities of growth and/or endocrine and neurological-behavioural functions (imprinting disorders) are associated with DNA methylation defects of imprinted genes, usually at a single locus. However, a subset of patients affected by imprinting disorders is characterized by multi-locus imprinting disturbances (MLID). Loss-of-function mutations affecting components of the sub-cortical maternal complex (SCMC) have been found in healthy women with reproductive problems and/or offspring with variable imprinting disorders. The mechanisms by which SCMC variants result in DNA methylation abnormalities is unknown. Most of the knockout female mice for the SCMC genes are infertile and their embryos do not develop beyond the 2-cell stage. We generated a mouse line carrying a hypomorphic missense variant, in the PADI6 gene. We chose a variant that was previously identified in compound heterozygosity together with a truncating mutation in the mother of two siblings affected by Beckwith-Wiedemenn syndrome and MLID. The mouse model was generated with homologous recombination and ES cells blastocyst injection. So far, we investigated the fertility of the homozygous female mice, and found that they are unable to have viable offspring. We plan to analyse DNA methylation and gene expression genome-wide in the oocytes of the homozygous female mice and in their pre-implantation embryos. We believe this approach may help understanding the mechanism by which Padi6, and in general SCMC genes, affect genomic imprinting.

Investigating the causes of DNA methylation disturbances in the Beckwith-Wiedemann syndrome

PhD student: **Francesco Cecere**

Tutor: **Prof. Andrea Riccio** (andrea.riccio@unicampania.it); **Prof. Flavia Cerrato** (flavia.cerrato@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli studi della Campania "Luigi Vanvitelli", 81100 Caserta;
Istituto di Genetica e Biofisica 'A. Buzzati-Traverso', CNR, 80131 Napoli.

The Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder characterized by several clinical features and increased risk to develop pediatric cancers. The most common molecular lesions of BWS are DNA methylation changes of imprinted loci located at chromosome 11p15.5, with loss of methylation of the KCNQ1OT1-TSS:DMR (also known as IC2 LoM) being detected in ~50% of the patients. One third of the IC2 LoM patients display methylation disturbances at multiple imprinted loci (MLID) other than those of 11p15.5. Recently, we and other research groups have demonstrated that in a subset of the BWS-MLID patients the methylation defect is associated with maternal-effect loss-of-function variants of genes encoding components of the subcortical maternal complex (SCMC).

By performing exome-sequencing and methylation-arrays, we have now identified novel and rare maternal effect variants of the SCMC genes in the mothers of several further BWS-MLID patients of our cohort. In addition to PADI6, NLRP2 and NLRP5, which have already been associated with BWS, we found that in one case loss of function variants also affected the KHDC3L gene, found mutated only in women with recurrent hydatidiform mole so far.

Also, we report on a patient affected by BWS, who developed colorectal adenocarcinoma (CRC) at 27 years old. We performed genome-wide methylation analysis on DNA extracted from blood, as well as normal and neoplastic colon tissue. This analysis revealed methylation disturbances at imprinted and cancer-associated genes in either normal and tumor tissues.

Our findings increase the spectrum of molecular defects associated with imprinted gene deregulation and variable clinical outcomes and highlight the need of multiple approaches for more accurate molecular diagnosis in imprinting disorders.

Meta-analysis of human retinal transcriptome data: a powerful tool to gain insight into the organization of inherited retinal disease genes and to identify putative interactors

PhD student: **Karla Alejandra Ruiz Ceja** (k.ruiz@tigem.it)

Tutor: **Sandro Banfi** (banfi@tigem.it)

PhD cycle: Scienze Biomolecolari – **35° ciclo**

Affiliation: TIGEM

Inherited retinal diseases (IRDs) are a genetically heterogeneous group of visual disorders that cause the dysfunction or death of photoreceptor cells and that can lead to blindness. To gain insight into the genomic organization and transcript composition of 48 genes among the most frequently involved in IRD pathogenesis, we analysed publicly available RNA-seq datasets obtained from the human retina. A total of 161 bulk RNA-seq human retina data from non-visually impaired post-mortem donors were retrieved (Pinelli et al., PMID:27235414 and Ratnapriya et al., PMID:30742112). We generated a merged Observed Transcriptome to identify putative novel transcripts belonging to the above genes.Transcript expression levels were quantified by scaling TPM (Transcript Per Million) abundance estimates per sample (scaled TPM) and transcripts with < 1TPM were filtered out. We found more than 800 putative novel transcripts among the analysed 48 IRD genes. The latter were the result of a) partial intron retentions, b) exon skipping, c) exon extension and, less frequently, d) putative novel exon inclusions.Furthermore, we reconstructed the gene networks underlying the expression of the 48 analyzed IRD genes by performing co-expression analysis. I am focusing my attention on a subset of long noncoding RNAs (lncRNAs) that were found to be significantly co-expressed with the IRD genes: we are currently determining their tissue distribution. I believe that a better knowledge on the genomic organization of causative genes and the dissection of the gene networks underlying their expression could bring to a better knowledge of IRD pathogenic processes.

Multilayer Network Omic Integration

PhD student: **Valeria Policastro** (valeria.policastro@gmail.com)

Tutor: **Annamaria Carissimo** (a.carissimo@na.iac.cnr.it) and **Claudia Angelini** (claudia.angelini@cnr.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Nowadays, with next-generation sequencing, a massive amount of heterogeneous data has been produced leading difficulties in the interpretations of such information. The individual analysis of each data set will give only a particular view of a disease or a biological process of interest. In contrast, integrating all the data will widen and deepen the results giving a more global view of the entire system. My research is devoted to the development of an algorithm to integrate different omics types to give a global view of the actors who play a fundamental role in the onset and progression of a given disease or a biological process. The developed method is based on a multilayer network structure that gives the possibility to analyse any type of data. Under the assumption that the structure underneath the different layers has some similarity that we want to pull out in the integrated network, we generate a "consensus network" through an iterative procedure based on structure comparison. Differently from simpler network integration models focussing on individual edges, the procedure tries to preserve common higher-order structures of the original networks in the integrated one, i.e. the neighbours of each node (genes, patients…). Once obtained the consensus network, we compare it with the starting networks extracting "specific networks", one for each layer, containing peculiar information of the single data type not present in all the others. Network integration can be useful in different circumstances. For instance, it can be used as a meta-analysis of a collection of the same kind of data from different labs, the method helps to extract common information even if the data can slightly differ. Another example concerns the analysis of the same type of omics data from patients in different tumour stages. The consensus network of the different stages can aid to identify the biological processes involved in the tumour while the specific networks can point out the processes related to a particular stage. This can be done also with different omics data (genomics, transcriptomics, epigenomics), each providing different associations with the disease. Integrating them can be useful to better understand the implicated biological mechanism and to identify relevant biomarkers

Session 3:

Structure and Functions of Biomolecules

Ultrasonic-Assisted Synthesis of Peptide Nucleic Acids

PhD student: **Alessandra Del Bene** (alessandra.delbene@unicampania.it)

Tutor: **Prof. Anna Messere** (anna.messere@unicampania.it)

PhD cycle: **36°cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Peptidic Nucleic Acids are synthetic DNA/RNA mimetics that are considered for numerous applications, including the development of gene-specific therapeutic approaches, of molecular probes (FISH) and of radiopharmaceuticals for gene expression imaging in cancers. Unfortunately, their chemical synthesis still remains a challenge due to the side-reactions occurring in PNA assembly (i.e. N-acyl transfer of the nucleobases) and the aggregation of the growing PNA chain on the solid support (particularly of purine-rich sequences). To overcome these problems various approaches have been attempted, including alternative protecting groups and coupling reagents, ligation strategies and automated microwave-assisted synthesis. These synthetic strategies led to some improvements in terms of quality, yield and size of synthesized PNAs, but expensive reagents and lab equipments are required. In this respect, during my first year of PhD, I focused on the development of an ultrasonication-assisted Solid-Phase PNA synthetic strategy. The rationale behind this approach is provided by the encouraging results achieved with the use of ultrasonication in the peptide synthesis. Specifically, US-SPS-strategy of PNAs has been successful applied for the synthesis of a model 9-mer sequence, a 13-mer polypurinic telomeric sequence (Pu 61%) and the difficult-long anti-miRNA sequence (23mer).

Effects of Glutamate on *C. elegans* Spinal Muscular Atrophy models

PhD student: **Federica La Rocca** (federica.larocca@unicampania.it)

Tutor: **Elia Di Schiavi** (elia.dischiavi@ibbr.cnr.it)

PhD cycle: **36° cycle.**

Affiliation: IBBR, CNR, Via P. Castellino 111, 80131 Napoli; Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta.

Glutamic acid (L-Glu) is an α-amino acid that has critical roles in protein synthesis, metabolism, and nervous system development and function. Alterations in the amount of L-Glu were observed in several neurological disorders, including Spinal Muscular Atrophy (SMA), a neurodegenerative disease characterized by the loss of motorneurons. To investigate the roles of L-Glu in SMA I'm using *C. elegans* and I tested the effects of supplementing different concentrations of L-Glu on three models of SMA, that mimic the three different forms of severity of the disease. L-Glu supplementation had no effects on the neurodegeneration, tested as the death of neurons, while it worsened the locomotion defects. Interestingly, L-Glu concentration was able to rescue the growth defects of a severe SMA model in a dose-dependent manner, but no improvement in lifespan assay was observed. These data suggest that L-Glu treatment has a dose-dependent effect on growth and further studies are required to better understand its role in SMA. Our studies confirmed that *C. elegans* is an excellent model to test and identify neuroprotective compounds that could be used in the development of new therapies.

Effects of the change in buffer conditions and of the substitution of the structural ion on the folding mechanism of Ros87

PhD student: **Rinaldo Grazioso** (rinaldo.grazioso@unicampania.it)

Tutor: **Carla Isernia** (carla.isernia@unicampania.it)

PhD cycle: **34° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

The prokaryotic zinc finger protein Ros87 represents an interesting model to study to the influence of metal ions on the structure, folding mechanism and function of metallo-proteins. We have previously reported that it shows a bipartite folding/unfolding process in which a metal binding intermediate converts to the native structure through a delicate barrier-less downhill transition [1]. Downhill folding can be described as a single thermodynamic state involving a conformations ensemble that gradually loses structure with the decrease of protein stability. It has been predicted to be rather rare in nature as downhill folders miss an energetically substantial folding barrier that can protect against aggregation and proteolysis [2]. Additionally, downhill folding mechanisms were originally thought to occur only for proteins with particularly optimized native interactions, under strongly stabilizing conditions or when mutations occurred. Considering also that important variation in folding mechanism can be observed within families of proteins with similar fold and high sequence identity and for the same sequence by varying conditions, we here characterize the unfolding mechanism of Ros87 and of its mutants in different buffers with different pH and ionic strength [3]. We also explore the effects on the structure and folding mechanism of the structural metal ion replacement with Ni(II), Co(II) or Cd(II). Our characterization is conducted by applying an integrated approach that combines information collected using different spectroscopic and calorimetric techniques. We aim at contributing to the general discussion showing that, in metallo-proteins, downhill folding can be commonly found under a much wider range of conditions and coupled to other different transitions.

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Nanoplastics impact on 3 different human gut commensals

PhD student: **Marica Sassano** (marica.sassano@unicampania.it)
Tutor: **Gaetano Malgieri** (gaetano.malgieri@unicampania.it)
PhD cycle: **34° cycle**
Affiliation: Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", via Vivaldi 43, 81100 Caserta, Italy

Enviromental pollution caused by plastic and its fragments and the risks to animal and human health is a topic that received a worldwide attention. Micro-nanoplastics are small plastic particles that derive from the fragmentation of various types of waste. Bottles, cosmetic products, clothing in fleece and synthetic fabrics: all these everyday objects when released into the environment leave a trail of small and very small particles. According to numerous research, micro-nanoplastics can have an impact on human health especially through the gastrointestinal tract, as its presence in the digestive system could influence the tolerance and immune response of the gut by bioaccumulating or by promoting the transmission of toxic and pathogenic chemicals as well as by disturbing the health gut microbiota. The intestinal microbiota is the complex microbial community of the enteric tract consisting mainly of bacteria, as well as yeasts, parasites and viruses. Some bacterial strains of the intestinal microbiota perform numerous beneficial functions, among all, indigenous strains hinder the colonization of the intestine by new microbes, including pathogenic ones. This project aims to analyze, using biochemical, microbiological and chemical techniques, the influence of micro and nanoplastics on the intestinal microbial community. In particular, some of the main bacterial strains that constitute the intestinal bacterial flora, having several properties regarding human health (*Bifidobacterium breve*, *Lactobacillus plantarum*, *Lactobacillus ramnhosus*) have been grown *in vitro* in the absence and in the presence of plastics and the influences of the presence of these pollutants on the survival of bacteria, their ability to form biofilms and their metabolism verified by means of different techniques. Overall, our data demonstrate a clear influence of nanoplastic on bacterial metabolism and in turn on their capability to form biofilm: although nanoplastic seems to increase the growth of all the studied species, it clearly causes a switch from sessile multicellular (biofilm) to planktonic unicellular state (suspension).

Dissecting the interaction of DDX11 with Timeless, a fork-protection complex subunit

PhD student: **Diana Santos**

Tutor: **Francesca M. Pisani** (francesca.pisani@ibbc.cnr.it)

PhD cycle: **35° cycle**

Affiliation: Institute of Biochemistry and Cell Biology (IBBC), National Research Council (CNR), Via P. Castellino, 111, 80131 Naples, Italy

The faithful transmission of genetic information is essential for survival and requires an accurate duplication of the chromosomes by the replisome machinery from one generation to the next. Helicases are evolutionary conserved enzymes that use energy from nucleoside triphosphate hydrolysis to unwind nucleic acid secondary structures and generate single-stranded DNA/RNA. DDX11, a super-family-2 DNA helicase with a 5' to 3' directionality, is implicated in cancer development and linked to the rare hereditary disease Warsaw breakage syndrome. The fork-protection complex is composed by Timeless, Tipin, Claspin and AND-1 proteins that are conserved from yeast to mammals. They are all key players in genome stability maintenance with different functions in DNA replication. It was reported that DDX11 directly interacts with Timeless to preserve replication fork progression in stressful conditions. To identify the Timeless subdomains involved in DDX11-binding, co-immunoprecipitation experiments were carried out with Flag-tagged DDX11 in combination with different Timeless fragments. Using this strategy, it was demonstrated that Timeless has two DDX11-interacting sites: one corresponding to a putative loop located in the N-terminal portion and another one located in the C-terminal part of the protein. These results will be interpreted and discussed based on the structural information available for the two proteins.

Investigating molecular determinants of cancer by cutting-edges high resolution mass spectrometry technologies

PhD student: **Mariangela Valletta** (mariangela.valletta@unicampania.it)

Tutor: **Angela Chambery** (angela.chambery@unicampania.it)

PhD cycle: **34° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Cancer is a multifaceted disease which results from dysregulated cellular signaling networks. While genes are considered the master regulators of diverse cellular processes, proteins are the effectors that mostly trigger and regulate molecular events at the crossroad of health or disease. The complexity of cancer biology requires the collection of comprehensive information on tumor proteomes and secretomes. 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.In this work emerging high-resolution MS strategies have been used for delineating the protein profiling of selected tumor cancer cell models including colon cancer (CRC) and triple-negative breast cancer (TNBC). Quantitative nanoLC-MS/MS TMT isobaric labeling-based approaches have been applied to the investigation of molecular determinants of cancer resistance. 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 have delineated a molecular hallmark of Pentraxin 3 (PTX3) overexpression in a TNBC model, identifying High Mobility Group Box 1 (HMGB1) as a secreted molecular determinant involved in the modulation of the angiogenesis driven by PTX3 overexpression. Collectively, our results pave the way to identify alternative pathways regulating the balance between inflammatory and angiogenic signals in physio/pathological conditions. A better understanding of cancer resistance mechanisms may provide valuable insights to help design new therapeutic strategies for the treatment of cancer.

Prokaryotic and Eukaryotic zinc-finger proteins

PhD student: **Veronica Russo** (veronica.russo1@unicampania.it)

Tutor: **Paolo Vincenzo Pedone** (paolovincenzo.pedone@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

The zinc finger is one of the major structural motifs involved in eukaryotic protein-nucleic acid interaction and can also participate in protein-protein interactions. Transcriptional factors containing Cys2His2 zinc fingers are present also in prokaryotes.
During the first year I focused my research activity on MucR protein from B. abortus. It is a zinc-finger protein containing a prokaryotic Cys2His2 zinc-finger domain; it has been described as a transcriptional regulator of many virulence genes and we have shown that behaves as H-NS like proteins (1). On this line of research we are collaborating with Prof. Dame from Leiden Institute of Chemistry (University of Leiden) to finally demonstrate that MucR is able to structure the DNA of B. abortus.
In this second year of my research activity, I also focused my attention on the protein-protein interaction of human zinc finger proteins (2, 3). In particular we have been studying the BTB/POZ zinc-finger protein ZBTB2. This protein is involved in cell proliferation and human cancers and it has been also described as an ARF, p53 and p21 gene repressor as well as an activator of genes modulating pluripotency. With the aim to define the ZBTB2 interactome and to identify the different proteins that could co-operate with ZBTB2 in its different functions, we identified ZBTB2 protein partners in U87MG cell line by high-resolution mass spectrometry (MS) experiments. Our analysis reveals an interplay between ZBTB2 and chromatin remodeling complexes, particularly with NuRD complex (3).

Small molecules for the treatment of PMM2- CDG

PhD student: **Mariateresa Allocca** (mariateresa.allocca@unicampania.it)

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.

PMM2-congenital disorder of glycosylation (CDG) is characterized by defects in the enzyme phosphomannomutase2. Our group has shown that both α-glucose-1,6-bisphosphate (αG16) and βG16 are good pharmacological chaperones enhancing the PMM2 activity in vitro. Considering that the anomer αG16 is the endogenous activator of the enzyme, we are trying to promote G16 bioavailability to facilitate the residual PMM2 activity. The lipophilic αG16 (lipo-G16) derivative was conceived as a prodrug able to pass through the plasma membrane. Once ensured the molecule is not toxic, I analyzed its effect on PMM2-CDG fibroblasts via NMR-based metabolomics. The intracellular metabolites were extracted from the cells using chloroform/methanol/water (1/1/1) after the administration of lipo-G16. Analysis of 1H NMR data revealed some differences between treated versus control fibroblasts and I am currently investigating the significance of these results to evaluate the efficacy of lipo-G16 as a drug. It was also evaluated the in vivo effect of lipo-G16 by growth experiments in yeast models of PMM2-CDG. Other evolution experiments showed genetic mutations appearing beneficial to PMM2-CDG yeasts therefore the biochemical characterization of these mutants is ongoing.

Characterization of quinoin, type 1 ribosome inactivating protein from *Chenopodium quinoa* Willd seeds

PhD student: **Angela Clemente** (angela.clemente@unicampania.it)
Tutor: **Antimo Di Maro** (antimo.dimaro@unicampania.it)
PhD cycle: **36° cycle**
Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100-Caserta, Italy

Type 1 Ribosome Inactivating Proteins (RIPs) are rRNA N-glycosylases (EC: 3.2.2.22) mainly isolated from angiosperms, which remove a specific adenine of the Sarcin-Ricin Loop (known as SRL) in the 28S ribosomal RNA (1). This damage of the protein synthesis machinery is related to RIPs cytotoxicity, in turn triggering apoptosis pathway. Moreover, besides their toxicity, RIPs have antiviral activity against plant and animal viruses, are allergenic and have transforming activity (2). Given their toxicity, the presence of RIPs is investigated in edible plants, although several edible plants are not eaten raw, since cooking inactivates these toxins by denaturing them. In this framework, Landi at el. (2021) previously purified and enzymatically characterised a type 1 RIP, named quinoin (29-kDa) from quinoa seeds, known as functional food. Quinoin is cytotoxic toward BJ-5ta (human fibroblasts) and HaCaT (human keratinocytes) in a dose- and time-dependent manner and presents high thermostability (3). Therefore, in this scenario, considering the presence of this toxin in seeds and quinoa sprouts raw consumption, we: i) investigated the presence of quinoin in sprouts; ii) performed *in vitro* digestive pepsin-trypsin treatment; and iii) evaluated the possible cross-reactivity between quinoin and other type 1 RIPs isolated from Caryophyllales order.

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Development and optimization of natural-abundance NMR techniques for studying structure and dynamics of proteins in solution and peptide-ligand interactions on the surface of living cells

PhD student: **Clementina Acconcia** (clementina.acconcia@unicampania.it)

Tutor: **Luigi Russo** (luigi.russo2@unicampania.it)

PhD cycle: **36° cycle**

Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), University of Campania "L. Vanvitelli", 81100 Caserta, Italy.

The Function of bio-macromolecules is principally determined by both their three-dimensional structure and conformational plasticity. Proteins are inherently flexible systems showing a broad range of dynamics on time-scales from pico-seconds to seconds. Nuclear Magnetic Resonance (NMR) spectroscopy represents a powerful tool for studying both protein structure and dynamics in solution and within cellular environment. A complete ^{1}H, ^{15}N and ^{13}C NMR chemical shifts assignment as well as ^{15}N relaxation rates are indispensable for an accurate description of the structural and dynamical features of proteins and peptides by NMR. Here, we report the development and optimization of a series of 2D heteronuclear NMR experiments for investigating at atomic resolution the structure and dynamics of proteins/peptides as well as protein/peptide-ligand interactions by exploiting the natural isotopic abundance. To test our developed NMR methodologies we applied these novel experiments to describe the structural and dynamical details of Pigeon, Eurasian woodcock and chicken myoglobins and to explore the molecular determinates driving receptor-ligand interactions on living cells surface.

NMR characterization of specialized metabolites from natural sources: evaluation their potential antimicrobial and antibiofilm properties and Understanding the Molecular Basis

PhD student: **Francesca Guzzo** (Francesca.guzzo@unicampania.it)

Tutor: **Brigida D'Abrosca** (Brigida.dabrosca@unicampania.it)

PhD cycle: **36°cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Plants, bacteria and marine organisms have always been considered inexhaustible sources of a wide variety of specialized metabolites, each with its own chemical structure responsible of a specific biological effect [1]. Literature data report the anti-bacterial activity of several natural compounds involved in for treatment of infections by multidrug-resistant bacteria, including Pseudomonas aeruginosa (gram negative) and Staphylococcus aureus (gram positive), two human pathogens causing major concern [2]. Myrtaceae Juss. is a very large family of flowering plants, that are a valuable source of compounds with antimicrobial properties [3]. Based on the consideration we investigated Myrcianthes cysplatensis, Psidium oligospemum and P. friedrichsthalianum, three plants belonging to Myrtaceae family. In particular, their dried leaves were extracted with solvents at increasing polarity, in order to explore their potential antimicrobial activity by disk diffusion assay. The obtained results revealed an interesting antimicrobial activity of chloroformic and methanolic extracts of Myrcianthes cysplatensis and P. friedrichsthalianum. 2D-NMR investigations, provide preliminary knowledge on the chemical composition of this plant. Focusing on the M. cysplatensis through repeated chromatographic steps leading to isolation and characterization of triterpenoids.

Structural and biochemical analysis of FeS DNA helicases

PhD student: **Manil Kanade** (manil.kanade@unicampania.it; manil.kanade@elettra.eu)
Tutor: **Silvia Onesti** (silvia.onesti@elettra.eu)
PhD cycle: **36° cycle**
Affiliation: Elettra sincrotrone Trieste

Helicases containing an iron-sulphur cluster (FeS) play an important role in many essential cellular processes like DNA repair, replication and genome maintenance. A detailed picture of the architecture and mechanism of FeS helicases is still lacking, stressing the need to further study these proteins at the molecular and cellular level. RTEL1 (Regulator of Telomere Length 1) belong the FeS helicase family has a distinct role in DNA repair, homologous recombination, telomere metabolism, and DNA replication. RTEL1 mutations have been linked to genetic disease and cancer pre-disposition. Structural, biochemical and biophysical studies require large amounts of very pure protein, which is a challenge for this protein family. To increase the chances of success we cloned the catalytic N-terminal domain of human, *C. elegans* and *X. laevis* RTEL1. The catalytic domain of the *C. elegans* helicase was soluble and could be purified to homogeneity. The protein is monomer in solution, binds different DNA substrates with a preference for D-loops and bubbles and unwinds DNA, in a reaction that is dependent on the integrity of the FeS cluster. This work provides a framework for an extensive characterization of this helicase by further biochemical analysis and for structural studies.

Structural Analysis of Human Prion Proteins using NMR Methodologies

PhD Student : **Manoj Madheswaran**

Tutor : **Dr Roberto Fattorusso**

PhD cycle: **35° cycle**

Affiliation: DiSTABiF, University of Campania Luigi Vanvitelli, Caserta, Italy.

Prion diseases are a group of neurodegenerative diseases in which normal cellular prion protein PrP^{C} turns into the infectious form PrP^{Sc}. However, the function of PrP^{c} is still unknown, it is necessary to determine the interaction between PrP^{c} with other molecules. PrP^{C} is a cell surface protein expressed and has a physiological structure with a C-terminal globular domain (amino acids 127–231) and an N-terminal flexible tail (amino acids 23–121). The N-terminal tail consists of two charged clusters (CC1 and CC2), the octarepeat region (OR) and a hydrophobic domain (HD). Additionally, two N-glycosylation sites are located in the globular domain upstream of the sialylated GPI-anchor at the C-terminus. It has 253 amino acids with two beta sheets and three alpha helices in its structure. The known functions described for PrP^{C} cover a wide spectrum including ion balance homeostasis, metal ion intake (such as copper and zinc), control of cell proliferation and neural differentiation. We used Nuclear Magnetic Resonance (NMR) spectroscopy which is one of the most powerful tools for biologists and chemists to study the structure and interaction of biological molecules. This project aims to analyse the function of cellular prion protein and its function under cellular mimicking environment by adding inert ficoll, an inert crowding agent. We identified that prion sample with ficoll reproduces the in-cell stability and kinetics of protein. This combination helps us to study interactions such as folding, binding, thermodynamic and kinetic of PrP^{C}. Bacterially expressed $HPrP^{C}$ used in NMR studies lack glycans during our NMR experiments we also monitored near glycosylation condition at positions Asn 181 and Asn 197. Since these positions are very important as they likely to protect PrP^{C} from proteases. We also performed Circular dichroism (CD) spectroscopy to analyse secondary structure and monitored the protein structural changes at various temperature.

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Impact of nano-plastics on the structure, dynamics and function of (bio)macromolecules and biological systems

PhD student: **Maria della Valle** (maria.dellavalle@unicampania.it)

Tutor: **Roberto Fattorusso** (roberto.fattorusso@unicampania.it)

PhD cycle: **35° cycle**

Affiliations: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta.

Istituto per i Polimeri, Compositi e Biomateriali, CNR, Via Campi Flegrei 34, 80078, Pozzuoli NA.

The plastics crisis, which is one of the major environmental changes, has gained worldwide attention in last years. Plastic micromaterials, easily, fragment in smaller particles, the nano-plastics, that we can find mainly in the air, water, and food, becoming dangerous to humans. So, it is extremely important to understand if and how the nano-plastics interact with biological systems. In the frame of this project, we outlined the impact of polystyrene nanoparticles on the one of the best-known proteins, the human ubiquitin. We performed a structural and dynamical characterization of the protein through a multidisciplinary approach in which TEM (Trasmission Electron Microscopy) and CD (Circular Dichroism) data were integrated with high-resolution NMR (Nuclear Magnetic Resonance) methodologies. Overall, our data strongly indicate that the addition of nano-polystyrene to the ubiquitin induces structural perturbations and local conformational rearrangements, that activate aggregation processes. On the other hand, to demonstrate biological effects of these polymers on cellular metabolisms, in vitro ubiquitination assay was carried out, and structural and functional in cell studies are in progress in order to identify any kinds of interaction and accumulation in the tissues.

Elucidating the role of DDX11, the Warsaw breakage syndrome DNA helicase, at the DNA replication fork.

PhD student: **Mohammad Mahtab** (md.mahtab@ibbc.cnr.it)

Tutor: **Francesca M. Pisani** (francesca.pisani@ibbc.cnr.it)

PhD cycle: **36° cycle**

Affiliation: Institute of Biochemistry and Cell Biology (IBBC), National Research Council (CNR), Via P. Castellino, 111, 80131 Naples, Italy / Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Regions of the genome with the potential to form unconventional DNA structures (as R-loops, triplexes and G-quadruplexes) pose a frequent and significant impediment to DNA replication and must be actively managed to preserve genetic integrity. DDX11 (also named ChlR1) is an ATP-dependent DNA helicase with 5′ to 3′ directionality, belonging to the super-family 2 (SF2) DNA helicases. Bi-allelic mutations of the DDX11 gene cause a rare hereditary disease, named Warsaw breakage syndrome (WABS). The amino acid changes produced by the different pathogenic DDX11 missense mutations were reported. Most of them map within DDX11 conserved helicase motifs and the relevant mutations are expected to impair the enzyme catalytic functions. DDX11 mutation R140Q, which was described in a recently discovered WABS affected individual, is considered as a variant of unknown significance (VOUS), as it does not map in any conserved sequence motif of the DDX11 polypeptide chain. Thus, it remains unclear whether R140Q DDX11 is a penetrant pathogenic allele. I intend to investigate a potential role of a DDX11 conserved region close to residue R140 in DDX11 catalytic functions. To this end, I will produce DDX11 site-specific mutants in this region and test them in biochemical assays (DNA-binding and -helicase assays). Besides, I plan to carry out functional rescue analyses to assess whether the DDX11 variants of interest can complement the DNA repair and chromosomal structural abnormalities of CRISPR-Cas9 DDX11-knocked out cell lines. Along with this, I am also studying the physical and functional interaction between DDX11 and PCNA to explore its relevance to genome maintenance pathways.

Hyaluronic acid and its derivatives based multifunctional nanostructured devices in cancer therapy and regenerative medicine

PhD student: **Mario di Gennaro**

Tutor: **Assunta Borzacchiello** (bassunta@unina.it)

PhD Cycle: **35° cycle**

Affiliation: Istituto per i Polimeri, Compositi, e Biomateriali (IPCB-CNR)

In the biomedical field the development of novel multifunctional materials is considered one of the main strategies to improve therapeutic treatments, in order to guarantee patients better life condition. In fact, invasiveness, low efficacy and side effects are challenging problems in many therapies such as cancer treatments and regenerative medicine applications. In particular, great attention is reserved in literature to devices prepared with renewable natural origin sources, such as hyaluronic acid (HA) (1). HA is a hydrophilic, not sulfated glycosaminoglycan, naturally occurring in mammalian tissues, that performs different biological functions, among which the joint lubrication and the ability to bind the cell surface glycoprotein CD44, that is overexpressed in many kinds of tumor cells, are the most relevant (2). In addition, HA exhibits reactive functional groups, such as the glucuronic acid carboxylic acid, that can be easily grafted with molecules of pharmaceutical interest (3). In particular, the chemical modification of polysaccharides by using natural origin prodrugs is considered an effective strategy to prepare new materials with improved therapeutical efficacy (4). Curcumin, for instance, is a diarylheptanoid obtained from turmeric, known for its anti-tumoral, anti-oxidant, anti-inflammatory properties, whose employment is limited by its reduced water solubility (5, 6). Curcumin conjugation to HA was demonstrated to improve it water solubility ad its bioavailability, and to obtain an amphiphilic derivative that spontaneously arranges in micelles (7). Another relevant molecule of natural origin is folic acid, a water-soluble vitamin B that participate in DNA and RNA formation, and that was used in literature to decorate nanoparticles for cancer therapy, to exploit the overexpression of folate receptors in any kind of tumors, such as that to the brain (8). In this work HA derivatives were prepared by using curcumin and folic acid for the preparation of nanostructured devices for cancer therapy and regenerative medicine.

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Role of Allelopathy in the Success of Selected Invasive Plant Species in the Mediterranean Basin and Possible Applications

PhD student: **Joyce Rodriguez** (joyce.rodriguez@unicampania.it)

Tutor: **Monica Scognamiglio** (monica.scognamiglio@unicampania.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Allelopathy is the biochemical interaction among plants wherein a donor species releases chemicals which affect the growth and performance of other plants. Most studies on allelochemicals have been towards their potential as natural herbicides. However, understanding the role of allelochemicals in natural ecosystems is of crucial importance. The capability of plants to produce and release allelochemicals into the environment determines the plants distribution, interaction, and diversity, therefore it might be one of the mechanisms involved in the success of invading species. This project focuses on two plants species that are invasive in the Mediterranean area, *Ailanthus altissima* and *Robinia pseudoacacia.* We will focus on the identification of allelochemicals from these donor plants through NMR-based metabolomics and bioassays aimed at understanding the effects of each into selected receiving plants in both morphological and molecular level. After isolation of the putative allelochemicals, experiments will be designed in order to study their mode of action at molecular level. Understanding the mode of action of the identified allelochemicals will help us determine their role in plant succession and plant invasion in both native and non-native environments. Furthermore, it might lay the foundation for their possible applications as herbicides.

Development of biocompatible hyluronan-based materials as drug-carriers and implant systems for tissue engineering

PhD student: **Valentina Verdoliva** (valentina.verdoliva@unicampania.it; valentina.verdoliva@gmail.com)

Tutor: **Stefania De Luca** (stefania.deluca@cnr.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

The main purpose of our research is to develop hyaluronan-based (HA) material which could exhibit safe circulation, so that could be implemented as biocompatible drug carriers. HA, a negatively charged naturally occurring polysaccharide, has been extensively investigated for several biomedical applications, due to its unique advantages of non-toxicity, non-immunogenicity, biocompatibility and biodegradability. More recently, HA-based nano-materials have received great attention and have also been studied as self-assembled nano-particles for drugs delivery applications. Therefore, we planned to prepare HA amphiphilic conjugates to investigate their ability to form nano-particles in an aqueous environment. Naturally occurring fatty acids were chosen as hydrophobic moieties to be conjugated to the hydrophilic HA skeleton, whereby allowing the resulting polymeric nano-system to imbibe only poorly water soluble drugs in their hydrophobic inner cores.We also planned to investigate the ability of the obtained HA-based material to generate hydrogels with the objective of developing a good Implant system for tissue regeneration.The designed synthetic strategy should result an energy-efficient and eco-friendly process. The aim is to tune a protocol that, starting from materials (hyaluronic acid and fatty acids contained in vegetable oils) provided by natural resources, is fully respectful of the GREEN CHEMISTRY basic principles. In addition, it is expected that the synthetic biopolymer (HA-fatty acid conjugates) can degrade to nontoxic fragments before renal excretion.

Specialized metabolites as potential lead compounds for anticancer drug discovery

PhD student: **Giovanna Valentino** (giovanna.valentino@unicampania.it)

Tutor: **Antonio Fiorentino** (antonio.fiorentino@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

The ever-increasing occurrence of intrinsic and acquired drug resistance in anticancer therapy calls for the development of new chemotherapeutic agents to prevent or overcome this problem. In this context, plant specialized metabolites are a very useful resource. However, finding new active molecules from the crude extracts is often a complex and time-consuming procedure. In latest years, the development of metabolomics, together with advances in analytical tools, has allowed a significant improvement in the identification of bioactive metabolites from crude extracts. This approach has been applied to seven Asteraceae plant species from Mediterranean area. NMR-based metabolomics was used to chemically characterize the plant extracts, while biological assays performed towards five human colorectal cancer cell lines, two human pancreatic neuroendocrine tumor cell lines, and two human melanoma cancer cell lines were used to screen the biological activity of the extracts enriched in specialized metabolites obtained by SPE. Multivariate data analysis of the results allowed us to identify *Centaurea deusta*, *Chondrilla juncea*, *Bellis sylvestris* and *Bidens subalternans* as the most promising extracts. Furthermore, it was possible to select a set of putative active compounds which will be isolated using a target approach to validate the biological activity and to understand the molecular mechanism.

Innovative mass spectrometry-based strategies for unraveling the Macrophage migration inhibition factor (MIF) interactome

PhD student: **Vikram Pratap Singh** (vikrampratap.singh@unicampania.it)

Tutor: **Angela Chambery** (angela.chambery@unicampania.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Macrophage migration inhibitory factor (MIF) was originally identified in the culture medium of activated T lymphocytes as a soluble factor that inhibited macrophage migration. MIF is a highly conserved 12.5-kDa protein that exhibits a unique combination of hormone-like, cytokine-like, and thioredoxin-like properties and is now recognized as a multipotential cytokine involved in the regulation of immune and inflammatory responses. A variety of cell populations have been shown to express and secrete MIF, suggesting that MIF is involved in a wide array of physiological and pathophysiological processes. It is imperative to understand the interaction network of MIF protein. The aim of this project will be the elucidation of the MIF interactome by applying a proximity-dependent Biotin Identification (BioID2) approach coupled to nanoLC- high resolution tandem MS. The identification of factors interacting with MIF may open novel perspectives for elucidating MIF's pleiotropic functions potentially relevant in cancer and other related diseases.

Exciton and charge separation: computational models

PhD student: **Haritha Asha** (haritha.asha@ibb.cnr.it)

Tutor: **Roberto Improta,** robimp@unina.it

PhD cycle: **34rd cycle**

Affiliation: Institute of Biostructure and Bioimaging, CNR

Excitonic Hamiltonians are widely employed cost-effective tools to model photoactivated processes in multichromophoric arrays. We have developed a Fragment Diabatization based Excitonic (FrDEx) model, a general and flexible approach, which incorporates charge transfer states. Using this FrDEx model we have computed the electronic circular dichroism and absorption spectra of two Guanine Quadruplex sequences (GQs), i.e., a fragment of the human telomeric sequence (Tel21, antiparallel), and $(TGGGGT)_4$ (TG4T, parallel). The results are fully consistent with the experimental spectra and in good agreement with that provided by the quantum-mechanical method used for the parametrization of FrDEx model. When applied to different structures generated by molecular-dynamics simulations on a fragment of the human telomeric sequence (Tel21/22), we found that ECD spectrum is moderately sensitive to the conformation adopted by the bases of the loops and more significantly to the thermal fluctuations of the guanine tetrads. In particular, we show how changes in the overlap of the tetrads modulate the intensity of the ECD signal. We illustrate how this correlates with changes in the character of the excitonic states at the bottom of the La and Lb local excitation bands, with larger involvement of local excitation and charge transfer states of bases that are more closely stacked.

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Session 4:
Molecular Cell Biology

Prolyl 3-hydroxylase 2 is a molecular player of angiogenesis.

PhD student: **Paola Pignata** (paola.pignata@unicampania.it/Pa.Pignata@gmail.com)

Tutor: **Sandro De Falco** (sandro.defalco@igb.cnr.it)

PhD cycle: **34° cycle**

Affiliation: Institute of Genetics and Biophysics Adriano Buzzati Traverso-CNR, Napoli. Via Pietro Castellino, 111, 80131 Napoli.

Prolyl 3-hydroxylase 2 (P3H2) catalyzes the post-translational formation of 3-hydroxyproline on collagens, mainly on type IV. Its activity has never been directly associated to angiogenesis. We identified P3H2 gene through a deep-sequencing transcriptome analysis of human umbilical vein endothelial cells (HUVECs) stimulated with vascular endothelial growth factor A (VEGF-A). We demonstrated that P3H2 is induced by VEGF-A in two primary human endothelial cell lines and that its transcription is modulated by VEGF-A/VEGF receptor 2 (VEGFR-2) signaling pathway through p38 mitogen activated protein kinase (MAPK). Then, we demonstrated that P3H2, through its activity on type IV Collagen, is essential for angiogenesis properties of endothelial cells in vitro, by performing migration and capillary sprouting in gain- and loss-of-function experiments. Immunofluorescence studies showed that the overexpression of P3H2 induced a more condensed status of Collagen IV, accompanied by an alignment of the cells along the Collagen IV bundles, so towards an evident pro-angiogenic status. Finally, we found that P3H2 knockdown prevents pathological angiogenesis in vivo in the model of laser-induced choroid neovascularization that recapitulate the wet form of age-related macular degeneration. Together these findings reveal that P3H2 is a new molecular player involved in new vessels formation and could be considered as a potential target for anti-angiogenesis therapy.

Exploring the role of the FANCJ DNA helicase in counteracting replication stress

Name**: Ana Sofia Cabaço Boavida** (anasofia.cabacoboavida@unicampania.it)

Tutor: **Francesca Pisani** (fm.pisani@ibbc.cnr.it)

PhD cycle: **35º cycle**

Affiliation: Consiglio Nazionale delle Ricerche – Istituto di Biochimica e Biologia Cellulare (CNR - IBBC)

DNA replication is a complex process that takes place each time a cell divides and several enzymes, like DNA helicases and DNA polymerases, are required for this process. DNA helicases have roles in several pathways of DNA metabolism and besides DNA replication they are also involved in DNA repair, recombination, transcription and telomere maintenance. FANCJ, a super-family 2 DNA helicase with an iron-sulfur cluster (Fe-S), unwinds duplex DNA with a 5' to 3' directionality in an ATP-dependent reaction and is associated with the ongoing replication forks. Unpublished data from our laboratory, demonstrated that human FANCJ interacts with AND-1, a DNA polymerase α-binding protein (discovered in budding yeast – Ctf4), and this interaction is mediated by a conserved sequence motif (CIP box). If critical residues in this putative CIP box are substituted with Alanine to create the FANCJ AALA mutant, binding to AND-1 is abolished both in vitro and in cell extracts. The FANCJ AALA mutant has helicase activity comparable to FANCJ wild type and can be considered as a separation-of-function mutant as it is helicase-proficient but AND-1-binding deficient. Preliminary data have revealed that FANCJ associates to the ongoing replisomes through a direct binding to AND-1 mediated by the CIP-box To perform most of the experiments, I produced stable cell lines expressing Flag-tagged FANCJ wild type and AALA mutant with a Doxycycline inducible system. I analyzed DNA damage response, in untreated and treated conditions by visualization of γ-H2Ax foci, performed SIRF assays, and co-immunoprecipitation experiments. I also started the functional analysis of FANCJ cancer relevant mutants to test their specific binding to AND-1 and the potential consequences on genome stability. Cellular localization of the FANCJ protein is also being analyzed throughout the cell cycle using immunofluorescence techniques.

The long non-coding RNA SPACA6-AS1, miR-125a and its mRNA targets establish a novel ceRNA regulatory network in hepatocarcinoma cells.

Name: **Armando Di Palo**

Tutor: **Nicoletta Potenza** (nicoletta.potenza@unicampania.it), **Aniello Russo** (aniello.russo@unicampania.it)

PhD cycle: **34º cycle**

Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta, Italy.

Non-coding RNAs play a crucial role in the onset and progression of hepatocellular carcinoma (HCC). During my PhD period, we investigated the roles of three non-coding RNAs, two oncosuppressive miRNAs (miR-125a and Let-7e) and a long non-coding RNA (SPACA6-AS1), all transcribed from the same genomic locus. SPACA6-AS1 is transcribed in opposite direction and its first exon sequence carries complementary sequences to miR-125a and miR-let-7e ones**.** We firstly validated the binding of the miRNAs to SPACA6-AS1; then over-expression experiments in HuH-7 and HepG2 cells showed the reciprocal inhibition between these non-coding RNAs. SPACA6-AS1 over-expression, in particular, could downregulate the miR-125a and let-7e expressions with the subsequent overexpression of their oncogenic targets in HCC (Lin28b, MMP11, SIRT7, Zbtb7a, Cyclin D1, CDC25B, HMGA2). These expression data, together with cell proliferation assays, demonstrated that SPACA6-AS1 could counteract the antiproliferative action of the two miRNAs. We also explored the expression data from The Cancer Genome Atlas (TGCA): in HCC samples SPACA6-AS1 are upregulated and miR-125a has a reverse expression compared to normal liver tissue, while for Let7-e this inverse expression was not observed. The data depict a novel competing endogenous RNA (ceRNA) network, ceRNET, whereby miR-125a can regulate the expression of SP-AS, which in turn regulates the miRNA by preventing its inhibitory binding to the target transcripts; in this regulatory circuit each RNA (miRNAs, lncRNA and mRNAs) can compete with others for their respective binding sites and the misleading of this balance could contribute to HCC**.** Intriguingly, an additional regulatory loop involving miR-125a and the oncogene Zbtb7a has been identified as a potential contributor to HCC. Finally, we detected a prevailing localization of SPACA6-AS1 into the nucleus, indicating a shuttling of the lncRNA. The functional significance of the nuclear localization is under investigation.

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

PhD student: **Chiara Siniscalchi** (chiara.siniscalchi@unicampania.it)

Tutors: **Aniello Russo** and **Nicoletta Potenza**

(aniello.russo@unicampania.it, nicoletta.potenza@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli"-81100 Caserta

My PhD project aims at investigating microRNA role in X-chromosome aneuploidy syndromes, i.e Turner and Klinefelter syndromes, wherein no obvious karyotype-clinical traits relationship exists. Genomic distribution analysis revealed the highest density of miRNA sequences on the X chromosome; this evolutionary conserved mammalian feature equips females with a larger miRNA machinery than males. However, miRNAs contribution to different X-related conditions, properties or functions is still poorly explored. For this purpose, the project is intended to accomplish a very large platform of miRNoma data for multiple comparisons aimed at identifying miRNAs involved in clinical traits of the syndromes, followed by functional analyses of selected miRNAs. First, a fine map of miRNA sequences on the X chromosome and possible modulators of their expression has been established, and bioinformatics functional analyses of the whole X-linked miRNA targetome were performed. Then, blood samples, adipose and muscle biopsies from Turner syndrome and Klinefelter syndrome adult and pediatric patients and healthy volunteers have been collected. In detail, during this year we have gathered a wide collection of blood samples from pediatric patients affected by both Turner and Klinefelter syndromes and relative age-matched healthy volunteers and we have extracted RNA. Next step in the workflow will involve miRNAseq analyses, identification of miRNAs differentially expressed, experimental target validation by cell cultures experiments; back to patient cohorts to analyze possible reverse expression correlation of miRNA-targets, further validating the functional role of those regulatory networks. This study could correlate miRNA-target networks to specific clinical traits to make sufficiently meaningful karyotype-phenotype associations, with important implications in terms of diagnostics and predictive medicine and development of innovative therapeutic approaches. Paralleling the described study, I also contributed to find human microRNAs interacting with SARS-CoV-2 RNA sequences by computational analysis and experimental target validation.

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

PhD student: **Giada Onorato** (giada.onorato@unicampania.it)

Tutor: **Elia Di Schiavi** (elia.dischiavi@ibbr.cnr.it)

PhD cycle: **35°cycle**

Affiliations: IBBR, CNR, Via P. Castellino 111, 80131 Napoli; Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta.

Neurodegenerative diseases, including Parkinson (PD), are determined by genetic and environmental factors. PD, characterized by the loss of striatal dopaminergic neurons, affects more often men than women. Male predominance was not observed in patients carrying G2019S mutation in *LRRK2,* one of the genes most frequently involved in PD. This more equal distribution between sexes is not limited to *LRRK2* and is shared by other genetic forms of PD. However how this genetic variation is causative or can modulate the PD-linked neurodegeneration remains unknown. To understand the molecular mechanisms causing the sex-specific differences in PD, I used two *C. elegans* PD models: the first overexpresses α-synuclein, a protein that can misfold and polymerize to form toxic fibrils coalescing into pathologic inclusions; the second overexpresses the G2019S mutated form of LRRK2. On both models, I demonstrated that sex can modulate the neurodegeneration, in an age-dependent manner. Then, using *LRRK2*^{G2019S} model, I investigated the genetic cues of sex-specific neurodegeneration and I found that dafachronic acid pathway is involved in the LRRK2^{G2019S} mediated sex-specific neurodegeneration. These results allowed us to identify the molecular mechanisms causing the sex-specific differences in a *C.elegans* model of PD and suggest new therapies for differential treatment of PD patients.

Role of D-aspartate oxidase on brain development and in neurodevelopmental disorders

PhD student: **Martina Garofalo** (martinagarofalo1994@gmail.com)

Tutor: **Alessandro Usiello** (usiello@ceinge.unina.it , alessandro.usiello@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania, " L. Vanvitelli", Via A. Vivaldi 43, 81100, Caserta, Italy. CEINGE Biotecnologie Avanzate, Via G. Salvatore, 482, 80145, Naples, Italy.

The *D-aspartate oxidase* (*DDO*) gene encodes for the catabolizing enzyme of D-aspartate (D-Asp), a free D-amino acid that occurs in mammalian brain at high concentrations in the embryonic phase and decreases after birth. D-Asp stimulates glutamatergic NMDA and mGlu5 receptors. Previous works reported the alteration of D-Asp metabolism with neurodevelopmental disorders such as Schizophrenia and Autism Spectrum Disorder (ASD). During the second year of my PhD project, we focused our attention on the still unknown role of precocious D-Asp occurrence on brain morphology and functioning. To clarify this issue, we generated a knock-in mouse model in which *Ddo* is overexpressed starting from the zygotic stage, to remove D-Asp in prenatal and postnatal life. Next, we translated our study from mouse to human. We found a clinical case of severe intellectual disability and ASD-related symptoms associated with duplication of *DDO* gene. Biochemical analysis of the patient's serum revealed that the duplication of *DDO* gene reduced the ratio of D-Asp versus total aspartate as compared with related controls. In conclusion, the patient's neuropsychiatric profile combined with abnormalities observed in the mouse model underline a key role for D-Asp metabolism in the regulation of neurodevelopmental processes associated with early glutamatergic transmission.

Short-Term Fructose Feeding affects insulin signaling and lipid metabolism by modulating microRNAs expression differently in young and adult rats

PhD student: **Giuseppe Petito** (giuseppe.petito@unicampania.it; giuseppepetito1993@gmail.com)

Tutor: **Antonia Lanni** (Antonia.lanni@unicampania.it)

PhD cycle: **34° cycle**

Affiliation: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "L. Vanvitelli", 81100 Caserta, Italy

In the last years, the dietary habits have changed worldwide, and the consumption of fructose has increased significantly. Numerous studies have provided compelling evidence that use of fructose can lead to impaired metabolic state and "has critical adverse effects". Recently, the role of miRNAs in the effects induced by high fructose consumption has been considered. In the light of the above, the aim of this study was to compare the metabolic perturbation induced by short-term fructose overconsumption (2weeks) in young rats (30 days) and adult rats (90 days). Most notably, the present study has focused on the miRNAs that could be related to the fructose detrimental effects. In young and adult rats fructose administration modulates differently the expression levels of miR122 and miR34a and their downstream targets in liver, skeletal muscle, white adipose tissue and serum. In addition, the hepatic expression levels of miR125b-5p are also modified in fructose-treated rats. These results show that adult rats have a greater susceptibility to metabolic perturbation induced by high fructose diet. In conclusion, the dysregulated miRNAs assemble as a regulatory network that cooperatively targets insulin signaling and lipid metabolic pathways contributing to pathogenesis of fructose-induced insulin resistance and non-alcoholic fatty liver disease (NAFLD).

Effect of ketone bodies and BDNF on thyroid hormone action in cerebral cortex of rats subjected to exercise and fasting.

PhD student: **Roberta Simiele** (Roberta.simiele@unicampania.it)

Tutor: **Pieter de Lange** (pieter.delange@unicampania.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli studi della "Campania Luigi Vanvitelli", Caserta, Italy

Physical exercise, related to nutritional interventions, elicits several beneficial effects at the central level. Exercise in fact plays a neuroprotective role on damaged neurons in diseases such as Parkinson's disease and Alzheimer's disease and also plays a protective role from oxidative stress. Furthermore, associated with fasting conditions, physical exercise causes a metabolic switch through the use of fatty acids as fuel and forces the brain to adapt to the use of ketone bodies as source of energy. The increase in ketone bodies and therefore in beta-hydroxybutyrate (BHB), induced by fasting, involves the activation of the brain-derived neurotrophic factor (BDNF). The peripheral beneficial effects of exercise can also be mediated by BDNF. Previous studies have led to the hypothesis that T3 acts as an endogenous mimetic of exercise and it has been shown that in rats, the fasting-exercise combination leads to normalization of serum levels of fT4 and an increase in BHB with subsequent up-regulation of BDNF in muscle.We study in detail how a period of fasting and exercise influenced the expression of BDNF and how it affects the signaling pathways downstream of BDNF in the prefrontal cortex. The results concerning the expression of genes that respond to T3 in various metabolic conditions, the levels of BHB and thyroid hormone in serum and prefrontal cortex will also be presented.

Direct effect of ketone bodies and thyroid hormone on BDNF maturation in muscle cells.

PhD student: **Arianna Cuomo** (arianna.cuomo@unicampania.it)

Tutor: **Pieter de Lange** (pieter.delange@unicampania.it)

PhD cycle: **35°cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, University of Campania Luigi Vanvitelli, Caserta, Italy

Lifestyle is a crucial element to counteract several diseases, in particular exercise and nutritional interventions have been shown to be activators of metabolic pathways that have beneficial outcomes. The potential beneficial effects derived from the combination of these conditions has been extensively studied in both animal models and humans. It is now known that dietary restriction with physical exercise causes rapid metabolic adaptations, especially in the skeletal muscle, and improves body. The fasting-induced reduction in fat mass is accompanied by the exercise-induced preserves of lean mass and muscle strength. Assuming that thyroid hormone is an important stimulus for the response to exercise and can be considered a mimetic of the latter, we observed that in rats subjected to short periods of fasting and exercise there is an increase in beta hydroxy butyrate (BHB) levels. Previous studies have shown that in these conditions, the brain derived neurotrophic factor (BDNF), reactive to BHB, was up-regulated at the transcriptional level in the muscle. We therefore created in vitro experimental model using L6C5 rat muscle cells, relating the effect of T3, BHB on BDNF transcription, translation and maturation, in presence and absence of glucose. We studied how these events affected cellular oxygen consumption.

PARP12 as a Novel Target in Cancer Resistance to Chemotherapy

PhD student: **Anupama Pavithran** (anupama.pavithran@ibbc.cnr.it, a.pavithran@ieos.cnr.it)
Tutor: **Dr. Giovanna Grimaldi** (giovanna.grimaldi@ibbc.cnr.it; g.grimaldi@ieos.cnr.it)
PhD cycle: **35th cycle**

Affliation: Department of Biomolecular Sciences, Institute Experimental Endocrinology and Oncology (IEOS), CNR,Naples

PARP12 is a mono-ADP-ribosyltransferase of the PARP family, with regulatory roles in membrane trafficking and cellular stress response [1,2]. Along with these functions, PARP12 has been identified as a key factor in breast cancer resistance to chemotherapy by contributing to tumour survival and re-growth [3]. To evaluate this further, we studied the PARP12 depletion effects in breast cancer cell lines in comparison to their non-tumorigenic counterparts. Further apoptosis induction upon PARP12 transient depletion in several cancer cell lines of different origin were also investigated by the detection of PARP1-cleavage by western blotting. Significant results demonstrated that transient depletion of PARP12 promotes apoptosis selectively in breast tumoral cells, as detected by FACS analysis and PARP1 cleavage. Interestingly, we found that Akt, a major regulator of cell survival [4], is a PARP12 substrate and that, upon PARP12-mediated ADP-ribosylation, Akt catalytic activity is increased. Presently, we are investigating this aspect on the protein p21, a well recognised Akt substrate. Further, by exploiting the ADPredict tool, putative Akt ADP-ribosylation defective mutants have been identified, that are currently under validation. In addition, we are generating PARP12 knockdown cell lines through lentiviral-based inducible shRNA system to further validate PARP12 sensitivity and tumour induction in *in vivo* models. These studies will improve our knowledge in understanding the enzymatic role of PARP12 in breast cancer resistance to chemotherapy.

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Patient specific cell lines as a model for Parkinson's disease associated to the gba variant e326k

PhD student: **Alessia Casamassa** (a.casamassa91@gmail.com)

Tutor: **Massimo Carella; Jessica Rosati** (email: m.carella@operapadrepio.it; j.rosati@css mendel.it)

PhD cycle: **35° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Heterozygous mutations in the GBA gene, encoding the lysosomal enzyme glucocerebrosidase, are the strongest known genetic risk factor for Parkinson's disease (PD), but the mechanisms underlying the increased PD risk and the variable phenotypes observed in carriers of different GBA mutations are not yet fully elucidated. The aim of my PhD project is to investigate molecular mechanisms and metabolic alterations underlying the association between PD and the GBA variant E326K through a multidisciplinary integrated approach based on cellular models. In the first part of the project we established primary fibroblast cell lines, necessary for the progress of the whole project. Fibroblasts, derived from the basal lamina of skin biopsies obtained from sporadic PD patients, patients carrying GBA mutation and healthy donors, were reprogrammed to hiPSCs through a *virus and feeder-free* approach; the hiPSCs clones were then characterized to evaluate their quality: we measured their gene expression, their capacity to produce embryoid bodies in vitro and teratomas in vivo, their karyotype and their Short Tandem Repeats (STR). Thus, we used an optimized protocol able to produce neurospheres from hiPSCs as neural model of this disease. In the second part of the project we dealt with the characterization of the GBA mutation, we analyzed both GBA gene and protein expression, its localization, also focusing on the state of cellular organelles. The last section of the project aimed to reveal whether there was a link between metabolic alterations of vitamin D_3, circadian cycle variations and the behaviour of these cells. The present study would be the first to document the relationship between alterations of vitamin D_3 metabolism and circadian rhythm and its role in determining the phenotype of neurodegenerative disorders, with particular attention to Parkinson's Disease.

Adiponectin protects against the neuronal damage induced by cerebrospinal fluid from multiple sclerosis *via* reducing oxidative stress and modulating INFγ expression

PhD student: **Marta Mallardo**

Tutor: **Aurora Daniele** (aurora.daniele@unicampania.it)

PhD cycle: **35°cycle**

Affiliations: Dipartimento di Scienze e Tecnologie Ambientali Biologiche Farmaceutiche, Università degli Studi della Campania Luigi Vanvitelli, Via G. Vivaldi 42, 81100 Caserta, Italy; CEINGE-Biotecnologie Avanzate, Via G. Salvatore 486, 80131 Napoli, Italy

Adiponectin (Acrp30), an adipose tissue-derived hormone, possesses multivalent biological functions both in physiological and pathological conditions. Recently, we showed that high Acrp30 levels in cerebrospinal fluid (CSF) are related to severity and prognosis of Multiple Sclerosis (MS), an autoimmune demyelinating disease of the central nervous system. Our purpose was to investigate: a) the effects of CSF from MS patients on U87 and SH-SY5Y cells, *in vitro* models of glioblastoma and neuroblastoma respectively, and b) to evaluate whether Acrp30 treatment interferes with the effects induced by CSF.To this aim, both cell lines were treated with CSF and/or Acrp30 and successively, cell viability, oxidative stress, and the expression levels of inflammatory mediators (IL-6, IL-10, TNF-alpha, INFγ) were evaluated by MTT, nitrite assay and Real time PCR. Our results demonstrated that MS CSF has cytotoxic activity on both cell lines that is moderately reversed by Acrp30 treatment on SH-SY5Y but not on U87 cells. The CSF toxic effects are partially mediated by oxidative stress, as demonstrated by induction of nitric oxide; again, Acrp30 is able to reduce release of nitric oxide induced by CSF treatment on SH-SY5Y cells but not on U87 cells. Finally, we found that CSF treatment induces the expression of INF□ on SH-SY5Y cells and that Acrp30 partially reverts this effect. Taken together, our data demonstrated that Acrp30 protects SH-SY5Y cells against MS CSF-induced cytotoxicity reducing the release of nitric oxide and modulating the expression of INFγ a major mediator involved in inflammatory response in MS.

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

PhD student: **Miriam Lucariello** (miriam.lucariello@ibbc.cnr.it; miriam.lucariello@ieos.cnr.it)

Tutor: **Carmen Valente** (carmen.valente@cnr.it)

PhD cycle: **35° cycle**

Affiliation: Istituto per l'Endocrinologia e Oncologia Sperimentale (IEOS)-CNR, Via Sergio Pansini, 5, 80131 Napoli NA; Istituto di Biochimica e Biologia Cellulare (IBBC)-CNR, Via Pietro Castellino, 111, 80131 Napoli NA.

CtBP1/BARS is a dual function protein: in the nucleus it acts as a transcriptional co-repressor regulating cancer progression, while in the cytoplasm controls membrane fission in several processes[1][2][3], including viral internalization via macropinocytosis[4]. Structurally, CtBP1/BARS possesses a Rossmann fold that upon binding to specific ligands[5] controls protein conformation and cellular functions. This mechanism underlies the "functional" molecular switch between the transcriptional and the membrane fission activities of CtBP1/BARS[5]. Using *Drug Repurposing* approach, starting from a library of approved "safe-in-man" drugs, we have validated 30 of them as selective inhibitors of CtBP1/BARS functions. Some of these drugs strongly impaired cell migration and invasion of two CtBP1/BARS-regulated tumours: melanoma and prostate cancer.

Moreover, the above 30 identified drugs have also been tested as potential blockers of macropinocytosis, the internalization pathway used by several coronaviruses including SARS-CoV-2 [6][7][8]. We have set-up an *in vitro* cell model in which the efficiency of the drugs in blocking SARS-CoV2 infection/internalization are under investigation in human ACE2 stable-expressing BHK21, using a VSV pseudovirus carrying the GFP-tagged Spike protein. Five of these drugs strongly impair virus internalization. Overall, these studies identified approved drugs, inhibitors of CtBP1/BARS cellular functions, that may be beneficial for cancer and viral infection therapies.

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Links between the autoregulation of apical cargo export from the TGN and the control of TNBC cell growth

PhD student: **Seyedehnegar Parizadeh** (seyedehnegar.parizadeh@unicampania.it)

Tutor: **Alberto Luini** (a.luini@ibp.cnr.it)

PhD cycle: **36° cycle**

Affiliation: Instituto di Biochimica e Biologia Cellulare, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

The secretory pathway responds to signals induced by the availability of environmental factors such as nutrients and growth factors. However, little is known about the internal regulation of the secretory pathway by signalling molecules. This project is dedicated to the characterization of auto-regulatory signalling at the TGN and to exploring its role in TNBC progression in 3D cell culture models and in vivo. The TGN is the main sorting station in the cells. Cargo proteins at TGN are destined to be sorted into either the basolateral or apical membranes or lysosomes. A signalling complex named ARTG (Autoregulation of TGN export) exerts a regulatory action on TGN export and sorting for basolateral proteins. In this project we focus on the organization and molecular determinants of the autoregulatory pathway of the TGN with a focus on apical cargoes. To this end, we will study the autoregulation of export of GPI-Anchored Proteins (AP). GPI-AP is one group of apical cargoes that are involved in many cellular processes. The presence of both lipid and protein in GPI-AP transport contributes to their unique properties. In spite of a good level of understanding of their organization and function, the important aspect of how they are regulated is still remained to be studied.

Session 5:
Human Genetics

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

PhD student: **Domenico Marano** (domenicomarano91@libero.it; domenico.marano@igb.cnr.it)

Tutor: **Floriana Della Ragione** (floriana.dellaragione@igb.cnr.it)

PhD cycle: **34° cycle**

Affiliation: Institute of Genetics and Biophysics "A.Buzzati Traverso", CNR, Via Pietro Castellino, 111, 80131, Italy

MeCP2 is an epigenetic modulator of transcription highly abundant in neurons and mutated in Rett syndrome (RTT), a neurodevelopmental disorder characterized by motor dysfunction, intellectual disability and autistic features, with unknown pathogenetic mechanism. Previously, we highlighted altered glycosphingolipid metabolism in *Mecp2*-null mouse brain, and upregulation of AUTS2, a key regulator of glycosphingolipid reprogramming, mutated in autism spectrum disorders. We aim to dissect the role of MeCP2/AUTS2 crosstalk in glycosphingolipid metabolism in a human neuronal context and to highlight a possible rescue of glycosphingolipid metabolism perturbations upon AUTS2 knockdown in a *MECP2*-null condition. To this purpose, we generated a novel *MECP2*-null SH-SY5Y human neuroblastoma cell line (*MECP2*$^{-/-}$) by CRISPR-CAS9 strategy. Selected clones and the wild-type counterpart were able to differentiate upon retinoic acid treatment and to express major brain gangliosides, such as GM1 and GT1b. We found altered levels of representative glycosphingolipids in *MECP2*$^{-/-}$ cells. Furthermore, we highlighted aberrant expression of genes encoding glycosphingolipid-related-enzymes (glycogenes) that parallels an unbalanced deposition of H3K27ac around their TSS. Moreover, AUTS2 was upregulated in the absence of MeCP2. Currently, we are inducing AUTS2 knockdown in *MECP2*$^{-/-}$ cells. In these cells, we will analyze large-scale glycosphingolipid content and will investigate the expression of selected glycogenes.

Hematopoietic differentiation of induced pluripotent stem cells (iPSCs) derived from patient with the Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome into HPCs expressing hematopoietic markers (CD34, CD43, CD45)

PhD student: **Barbara Morone** (barbara.morone@libero.it)

Tutor: **Maria R. Matarazzo**

PhD cycle: **34° cycle**

Affiliation: Institute of Genetic and Biophysics "Adriano Buzzati Traverso" (IGB-ABT), CNR, Naples

The ICF syndrome is a rare disorder causing immunodeficiency. Majority of patients carry mutations in DNA-methyltransferase-3B gene. Our aim is to investigate the early immunological defects occurrence studying the differentiation of iPSCs towards hematopoietic progenitor cells (HPCs), using a 12-days "in vitro" protocol. Specifically, we compared the results obtained from the differentiation of iPSC derived from fibroblasts of ICF1 patients, healthy donors (HDs) and isogenic ICF-iPSC clones in which specific mutations were corrected through CRISPR-Cas9 editing. Higher mortality at day 12 was clearly observed in ICF1 cells when compared to HD- and corrected ones. Annexin V staining suggests the involvement of apoptosis in this phenomenon. Moreover, the HPCs were analyzed by flow-cytometry for the expression of the hematopoietic cell surface markers CD34, CD43 and CD45. The HPC population obtained from ICF-iPSC resulted less enriched into cells expressing these hematopoietic markers if compared to HD-HPCs, suggesting that ICF-iPSCs are capable to generate HPCs in vitro but at lower level and with a pronounced mortality. In order to deeply evaluate the molecular differences between HPCs derived from ICF and HD cells, we are analyzing them through bulk RNA-seq experiments. Meanwhile, we are evaluating and setting reprogramming strategies of cells from blood (CD34+ and whole PBMC).

AAV-mediated microRNAs modulation as gene-independent strategy in inherited retinal dystrophies

PhD student: **Martina Di Guida** (martinadiguida@gmail.com)

Tutor: **Sandro Banfi** (banfi@tigem.it)

Co-tutor: **Sabrina Carrella** (carrella@tigem.it)

PhD cycle: **35°cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Inherited retinal dystrophies (IRDs) are a group of genetic disorders affecting the retina characterized by photoreceptor (PR) cell death and progressive vision loss, and include a variety of clinical subtypes, among which retinitis pigmentosa (RP). They are genetically heterogeneous with over 250 causative genes identified to date. For this reason, there is high need of gene-independent therapies that could be employed to delay degeneration and could be used in combination with gene-replacement strategies. In this respect, microRNAs (miRNAs) represent promising therapeutic tools due to their capability to simultaneously modulate multiple molecular pathways involved in human disease pathogenesis and progression. We observed that the modulation of miRNAs miR-181a/b or miR-204 preserves retinal cells from death and ameliorates visual function in the RHO-P347S mouse, a model for an autosomal dominant form of RP. We now tested our strategies in two models of autosomal recessive IRDs: rd^{10}, a mouse model of RP, and Abca4-/-, a mouse model for Stargardt disease. We observed that miR-181a/b downregulation ameliorates retinal function and morphology in rd10 mice and reduces toxic by-product accumulation in the retina of Abca4-/- animals, supporting the gene-independent protection exerted by this strategy. Additional data will indicate whether this strategy could have additive effect in combination with gene-replacement strategies.

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

PhD student: **Georgios Petrogiannakis** (georgios.petrogiannakis@unicampania.it; petrogiannakis.geo@gmail.com)

Tutor: **Sandro Banfi** (banfi@tigem.it)

Co-tutor: **Sabrina Carrella** (carrella@tigem.it)

PhD cycle: **35° cycle**

Affiliation: Telethon Institute of Genetics & Medicine (TIGEM), Via Campi Flegrei 34, 80078 - Pozzuoli (NA), Italy

Inherited retinal diseases (IRDs) are a clinically and genetically heterogeneous group of disorders, characterized by progressive photoreceptor degeneration and incurable loss of vision. MicroRNAs (miRNAs), a class of endogenously expressed non-coding RNAs with post-transcriptional regulatory properties, are known to play a major role in retinal function, both in physiological and pathological conditions. Since miRNAs are capable of simultaneously modulating multiple molecular pathways, they represent promising tools to therapeutically tackle disorders with high genetic heterogeneity such as IRDs. In the present work, I carried out a high-throughput screening approach to study miRNAs' impact on a photoreceptor cell line undergoing light-induced degeneration. For this analysis, more than 1200 miRNAs were transfected and assayed for their putative protective action in light-stressed 661W cone photoreceptor cells. Hereafter, the protective role of the top-ranked miRNAs is being confirmed individually with additional *in vitro* and *in vivo* methods, having as a final aim the unravelling of possible molecular mechanisms underlying their protective role. The identification of miRNAs exerting a relevant effect could shed further light about the process of photoreceptor degeneration that remains unclear and, furthermore, lead to the potential development of novel therapeutic approaches for IRDs.

Zfp687^{P937R}-knock in mouse model exhibits features of Paget's disease of bone and an altered bone marrow composition

PhD student: **Sharon Russo** (sharon.russo@unicampania.it)

Tutor: **Fernando Gianfrancesco** (fernando.gianfrancesco@igb.cnr.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta; Institute of Genetics and Biophysics (IGB-ABT) - CNR, Via Pietro Castellino 111, 80131, Naples.

Paget's disease of bone (PDB) is a late onset disorder of bone remodeling. The P937R germline mutation in the *ZNF687* gene is causative of a severe form of PDB complicated by Giant Cell Tumor. ZNF687 is a C2H2 zinc finger protein, known to act as transcriptional regulator. To uncover its functional role in PDB pathogenesis, we generated the knock-in mouse model, carrying the same mutation in the homologous *Zfp687* gene. μCT analysis performed on 3-month-old mice did not reveal bone alteration, neither in bone mass nor structure. Nevertheless, we histologically evaluated the osteoclast activity in the 3-month-old femora to assess the cellular activity. TRAP-staining revealed a significative increment of $TRAP^{+}$-osteoclasts in homozygous mice (n=5, p=0,0326) and a positive trend in heterozygous mice compared to the counterpart (n=5). Moreover, preliminary data showed an altered bone marrow composition in mutant mice, exhibiting an increase in the number and size of adipocytes equivalent to almost 1.5fold-enhancement, compared to the control. These results suggest not only an osteoblast-adipocyte imbalance, but also that Zfp687 could play a role, as transcriptional factor, in the lineage-commitment of skeletal stem cells.

Interestingly, 8-month-old heterozygous and homozygous mice showed a significant decrease in the trabecular bone volume to total volume ratio (BV/TV) in the femora up to 27% and 35%, respectively. Similarly, bone mass was reduced in L4 vertebrae of mutant groups up to24%. Additionally, femoral cortical lesions were found in mutants, implying PDB features.

Development of innovative diagnostic protocols for the prediction, progression and monitoring of Parkinson's disease

PhD student: **Giorgio Fortunato** (Giorgio.fortunato@unicampania.it)

Tutor: **Teresa Esposito** (teresa.esposito@igb.cnr.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta ; IGB- CNR "Adriano Buzzati Traverso" ,Via Pietro Castellino 111 - 80131 Naples - Italy

We recently identified 26 genes associated with late onset Parkinson's disease (LOPD) and demonstrated that the co-inheritance of multiple rare variants in these genes may predict PD occurrence in about 20% of patients, both familial and sporadic cases, with high specificity [1]. The objectives of this study are the identification of novel PD genes, by extending the whole exome sequencing (WES) analysis to additional PD families, and the study of the functional role of single and multiple mutations in PD-disease cellular models. In the last year we recruited 14 LOPD families and the WES in ongoing. We tested the pathogenicity of five mutations identified in the lysosomal K+ channel TMEM175 by patch clamp electrophysiology analysis and demonstrated a significant reduction of the potassium ion current associated with all the identified mutations. To study the interaction of multiple mutations in different genes, which is the genetic condition identified in a consistent number of patients, we started to collect fibroblast cell lines and peripheral blood mononuclear cells (PBMC) derived from PD patients and healthy subjects to generate induced pluripotent stem (iPS) cells to study the functional role of the different mutations on the identity and/or survival of dopaminergic neurons to neurodegenerative insults.

The role of miR-181 in Parkinson Disease

PhD student: **Romina D'Alterio**

Tutor**: Brunella Franco** (franco@tigem.it)
Co-tutor: **A. Indrieri** (indrieri@tigem.it)

PhD cycle**: 35°cycle**

Affiliation: Università Vanvitelli-TIGEM (**Telethon Institute of Genetics and Medicine**)

Parkinson's Disease (PD) is the second most common neurodegenerative disorder characterized by the progressive loss of dopaminergic (DA) neurons of the substantia nigra (SN) pars compacta. Mitochondrial dysfunction has a prominent role in neurodegenerative events, especially in PD. MicroRNAs are fine regulators of gene expression and their dysregulation in PD has been reported. miR-181a and miR-181b (miR-181a/b) are highly expressed in the SN and striatum and enriched in the brains of PD patients. In the past, we showed that mir-181a/b downregulation ameliorates neurodegeneration in mitochondrial disorders models. To test the effect of mir-181a/b modulation in PD, we generated chemical PD models in Medakafish and mice by using the neurotoxin 6-hydroxydopamine (6-OHDA) and, in both models, the inactivation of miR-181a/b reduces the extent of 6-OHDA-induced DA neurons death. Moreover, we are validating the neuroprotective effect of miR-181a/b modulation in *in vivo* and *in vitro* model of α-synucleinopathy PD. Finally, we are now evaluating if miR-181a/b could be considered as molecular biomarkers of PD. We are thus performing RT-qPCR to estimate miR181a/b levels in plasma of PD patients. In conclusion, our preliminary results suggest that miR181a/b may represent a both reliable and easy to measure biomarkers, and effective therapeutic targets in PD.

Genetics of oxidative stress in a population-based study

PhD Student: **Pasqualina Cennamo** (pasqualina.cennamo@unicampania.it)

Tutor: **Marina Ciullo** (marina.ciullo@igb.cnr.it)

PhD cycle: **35° cycle**

Affiliations: Institute of Genetics and Biophysics 'A. Buzzati-Traverso', CNR, 80131, Naples; Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), University of Campania 'Luigi Vanvitelli', 81100, Caserta.

Oxidative stress (OS) results from imbalance between excessive production of reactive oxygen species and limited antioxidant defenses[1]. In our study serum oxidant and antioxidant species have been assessed using commercially available tests from Diacron International (dRoms, BAP and anti-Roms tests) in a cohort consisting of individuals from three isolated populations. Genome-Wide Association Studies were performed to analyze the relationship between genetic polymorphisms in our sample and serum levels of oxidants and antioxidants. Significant signals have been identified for anti-Roms trait (rs4447862 in SLC2A9, P=6.91E-09; rs111741722 in UGT1A, P=7.95E-13). In accordance with capacity of this measure to detect uric acid and bilirubin as antioxidants, our results identified specific genes known to be associated with serum uric acid and bilirubin levels. Polygenic risk scores (PRS) provide a novel approach to estimate an individual's genetic disease risk by combining the effects of variation at multiple, functionally related genes[2]. Using our GWAS data of oxidant and antioxidant measurements, PRSs will be calculated and tested for association with OS-related phenotypes such as obesity-related traits and Alzheimer's disease. A pathway-based approach, constructing PRSs for sets of genes that encode components of OS-related pathways (mitochondrial functions), will be also investigated for association with the selected diseases.

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Genome-wide studies for the molecular characterization of isolated Wilms tumor

PhD student: **Abu Saadat** (abu.saadat@unicampania.it)

Tutor: **Andrea Riccio** (Andrea.RICCIO@unicampania.it)

PhD cycle: **36° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Wilms tumor (WT) is a rare childhood embryonal tumor affecting 1 in every 10,000 children worldwide. It is a renal tumor derived from the embryonal nephric mesenchyme The cause of WT is not clearly established but heredity is rarely a role player whereas DNA methylation defects at chromosome 11p15 are commonly reported. The region 11p15.5 consist of two clusters of genes expressed in a parent of origin-based manner implied through genomic imprinting controlled by epigenetic marks differentially established on the maternal and paternal alleles in the gametes or early embryo. The two clusters are regulated by two imprinting control centres: ICR1 (IGF2/H19 IG-DMR) controlling H19 and IGF2 genes, which is found hypermethylated in WT, and IC2 (KCNQ1OT1 TSS-DMR) controlling CDKN1C and KCNQ1OT1. Both clusters are affected by imprinting and methylation defects in WT with paternal UPD. There are other risk factors also involved including predisposition to WT among children with Beckwith Wiedemann syndrome, various mutations, and other chromosomal ablations.As previously reported by our group, both the epimutations and chromosomal aberrations affect theaggressiveness of WT. The main interests I would be addressing through my study in the upcoming years would be to understand and characterize the molecular bases of WT, to understand the molecular mechanism of imprinting dysregulation and its correlation with the aggressiveness of tumor progression. We will try to address the questions using NGS approaches for the identification of gene variants, methylation defects and transcriptional alterations of two large cohorts of Italian and Spanish sample.

Unsolved rare cases: towards new diagnostic strategies

PhD student: **Maria Elena Onore** (maria.elena.onore@gmail.com; mariaelena.onore@unicampania.it)

Tutor: **Vincenzo Nigro** (vinnigro@gmail.com)

PhD cycle: **34° cycle**

Affiliation**:** Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. De Crecchio 7, 80138, Naples, Italy

Although gene panels, WES or WGS analysis has changed our approach to the molecular diagnosis, many genetic conditions remain unsolved due to inability of NGS in recognizing small quantitative changes like intragenic deletions/duplications or large and complex genomic rearrangements. Until now, a large cohort of over 600 rare disease patients has been recruited for the Telethon Undiagnosed Diseases Program (TUDP). After WES, about 50% of them remained unsolved. For these cases, it is mandatory to identify new strategies to increase our diagnostic detection rate. To this aim, we used two different approaches: the 10X Linked-Read WGS technology and the engineering of a new quantitative high-resolution CGH-based test. The former combines single-molecule barcodes with short-read sequencing. The latter is an exome-based array CGH (ACACIA) with a full single-exon gene coverage. Altogether, they can be useful in defining large or complex genomic rearrangements and in identifying small intragenic copy number mutations (CNMs). Using 10X technology we were able to reveal a complex structural variant in a DMD carrier with an unsolved genetic state, thanks to phasing both X chromosomes. We also engineering ACACIA with probes covering 7,835 genes. Now, we are testing our design on sixteen selected unsolved pediatric cases (singletons) from the TUDP project. By integrating WES analysis with 10X Linked-Read WGS and/or ACACIA, we are confident to enhance the diagnostic detection rate for TUDP unsolved patients.

A pipeline for prioritization of putatively damaging genetic variants in cases of oocytes/embryo developmental arrest

PhD student: **Silvia Buonaiuto** (silvia.buonaiuto@unicampania.it)

Tutor: **Vincenza Colonna** (vincenza.colonna@igb.cnr.it)

PhD cycle: **34° cycle**

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Failure of embryo development can happen at several stages, namely maturation of the oocytes, fertilization, and before/after implantation. Mutations affecting genes involved in fundamental processes of oocyte fertilization and embryo development are candidates for unexplained infertility investigations. We developed the GP pipeline to prioritize putatively damaging genetic variants in coding regions, based on the prediction of the functional effect of the variants. Our pipeline incorporates prior information on genes involved in the trait under study but is also robust to the discovery of novel genes. We applied the GP pipeline in pre-and post-implantation phenotypes in women and embryos respectively. From the analysis of 22 maternal exomes, we identify 987 unique variants in 880 genes. Genome-wide functional validation revealed that 87.8% of the prioritized genes harboring high-impact variants are expressed by individual human mature oocytes and/or in the antral granulosa cells. From the analysis of the whole genome of 10 euploid miscarried embryos, we prioritized 439 putatively causative single nucleotide polymorphisms in 399 genes. Among the prioritized genes in the embryos we found *STAG2* for which inactivation in mice is lethal, and *TLE4* a target of Notch and Wnt, physically interacting with a region on chromosome 9 associated with miscarriages.