TITOLO E ABSTRACT TEMA DI RICERCA DOTTORANDI DEL CORSO DI DOTTORATO DI RICERCA IN MEDICINA MOLECOLARE E TRASLAZIONALE

XL ciclo (A.A. 2024/25)

Aqsa Anjum

Tema di ricerca: *Impact of maternal high fat diet during gestation on neurodevelopment in the offspring*

Abstract: Environmental factors are increasingly emerging as major players in the etiology of neurodevelopmental and psychiatric disorders, and among these factors is the exposure to adverse events during intrauterine life. A most harmful event in this respect is the maternal consumption of a high-fat diet (HFD) during gestation, which may impair brain development in the offspring. Indeed, clinical studies have shown that an increased incidence of neurodevelopmental/psychiatric disorders may exist in individuals born to mothers who consumed HFD during gestation. Clinical findings have been extended by studies in experimental animals, which showed that gestational exposure to maternal HFD may induce in the offspring behavioral anomalies that recall those featuring human neurodevelopmental and psychiatric disorders, along with altered function of dopaminergic, opioidergic, and serotonergic systems. The detrimental effects that gestational exposure to maternal HFD elicits on the dopaminergic neurocircuitries of the offspring are most notable, since these pathways, and particularly the mesocorticolimbic system, critically regulate emotional, social, and memory domains, which are impaired in neurodevelopmental and psychiatric disorders. While it has been reported that gestational exposure to maternal HFD may induce a transgenerational dysfunction of the dopaminergic mesocorticolimbic system, the events underlying this phenomenon are ill-defined.

The PhD student is involved in a project that will employ a multidisciplinary approach to characterize the anomalies in function, structure, and connectivity that the gestational exposure to maternal HFD may elicit in the dopaminergic mesocorticolimbic system of the offspring to understand how these anomalies may trigger behavioral impairments that recall those featuring human neurodevelopmental and psychiatric disorders. To this end, a rat model of gestational exposure to maternal HFD will be used to evaluate and stage in the offspring: i) the alterations in the emotional, social, and memory domains; ii) the molecular events that contribute to loss of dopaminergic synapses in the ventral striatum and prefrontal cortex and to neuronal malfunction in the mesencephalon; iii) how modifications in the connectivity within the dopaminergic mesocorticolimbic system and between this pathway and the cholinergic, noradrenergic, and serotonergic systems contribute to the behavioral impairments that stem from gestational exposure to maternal HFD. The PhD student will be involved in the (ii) aim by using different techniques (immunohistochemistry, ELISA, blot, PCR) under the supervision of her tutor, Prof. Cristina Cocco, to specifically identify those biomolecules affected by gestational exposure to maternal HFD in the offspring. These findings will have marked translational value, since dysfunctions in the dopaminergic mesocorticolimbic system are a major player in human neurodevelopmental and psychiatric disorders

Thonas De Berardini

Tema di ricerca: *Variations of metabolomic profile at different training modalities.*

Abstract: Lack of physical ac-vity and inadequate nutri-on are at the root cause of the onset of numerous non-communicable chronic diseases (NCDs), when pathology is almost overt, lifestyle changes, like nutri-on and exercise, are very important therapeu-c approach both to control symptoms and prevent worsening and reducing costs for the healtcare system; however, the lack of compliance makes this type of interven-ons very hard to realize in a real life scenario. In the female popula-on of childbearing age, the most common clinical scenario is that of a low-calorie diet and physical ac-vity limited to endurance training (ET); this situa-on, if prolonged over -me, leads to a well-known condi-on of reduced energy availability

(LEA) which makes it even more difficult for the organism to adapt to the s-muli of strength training (Resistance Training – RT), when present. The inadequate energy intake, due to wrong strategies and/or an underes-ma-on of daily caloric requirement, the lack of results, the product of a widespread underexposure to RT, determine a ""neuro- psychometabolic"" impairment whose main effects, related to each other, are:

1. Loss of mo-va-on;
2. Dropout from diet and physical ac-vity;
3. Lack of results;
4. Lack of scien-fic data.

From these assump-ons emerges the need to conduct a study that evaluates both caloric needs and physical ac-vity levels with precise methods, quan-ta-vely and qualita-vely, correla-ng different training methodologies with the possible efficiency of Metabolic Flexibility (MetF), especially with regard to the mixed mode (Concurrent Training – CT), a combina-on of ET and RT in the same session, in fact, this modality could be more effec-ve in changing metabolic flexibility both as a func-on of the -me of interven-on and as a func-on of its magnitude, thus making it eligible as the main modality in the prescrip-on of Adapted Physical Ac-vity in the case of NCDs, allowing greater compliance and providing more meaningful short- and medium-term outcomes."

Stefano Di Michele

Tema di ricerca: *Metabolic Flexibility as a Therapeutic Target in Polycystic Ovary Syndrome: Bridging Molecular Mechanisms and Nutritional Strategies*

Abstract: Metabolic flexibility is defined as the ability of an organism to adapt fuel oxidation to changes in nutrient availability and energy demand, thereby efficiently switching between carbohydrate and fatty acid utilization. In Polycystic Ovary Syndrome (PCOS), a condition frequently associated with insulin resistance and metabolic dysregulation, reduced metabolic flexibility has been implicated in the onset and progression of metabolic disturbances.

This study aims to investigate metabolic flexibility in a cohort of women with a new diagnosis of PCOS who have been identified as strongly insulin-resistant via an oral glucose tolerance test. At baseline, participants will undergo a comprehensive cycle ergometer protocol during which respiratory gas exchange ratio (RER) (carbon dioxide production/oxygen consumption) will be continuously measured using a facemask connected to a gas analyzer, to evaluate substrate utilization. Blood samples will be collected at five-minute intervals during the progressive exercise test to assess glycemia and lactate levels and several metabolic markers. These data will provide insight into substrate utilization, lipid metabolism dynamics, and specific circulating lipid biomarkers that reflect the body's capacity to use fatty acids as an energy source and the balance between PPAR-alpha activity and the endocannabinoid system, which regulates metabolic flexibility and is reflected by changes in the RER.

Following the baseline assessment, participants will begin a combined treatment of estrogen-progestin therapy and metformin. After a defined treatment period, the exercise test and biomarker analysis will be repeated to evaluate changes in metabolic flexibility. Based on individual therapeutic responses and metabolic profiles, a long-term personalized nutrition and physical activity program will be developed. Machine learning techniques will support the optimization of this personalized intervention. By correlating changes in respiratory parameters with biochemical markers and insulin sensitivity, this study aims to uncover the molecular mechanisms driving metabolic inflexibility in PCOS and to assess the effectiveness of a combined hormonal, metabolic, nutritional, and lifestyle intervention.

This multidisciplinary approach is poised to contribute valuable evidence toward optimizing treatment strategies for PCOS, potentially paving the way for targeted nutritional, lifestyle, and medical interventions that restore metabolic homeostasis and improve overall clinical outcomes."

Daniela Diana

Tema di ricerca: *Physiological and genetic factors underlying inter-individual variability of olfactory function in humans*

Abstract: In humans, olfactory function strongly influences the quality of life, playing an important role in eating behavior, in the ability to detect odors that signal the presence of danger (eg: gas, smoke, spoiled food), in social communication (reproductive behavior, mother-infant recognition, identification potential mating partners) and in personal hygiene. In particular, olfaction contributes to nutritional health and food pleasure by mediating the perception of food odors. Most people with olfactory deficits report that food is less tasty and less enjoyable, and these conditions subsequently modify their eating habits. In general, these individuals report an increase in the consumption of more palatable foods, such as sweet and high-fat foods compared to fruits and vegetables, as well as a greater use of condiments and spices, to compensate for the reduced gratification resulting from less olfactory stimulation and show a higher prevalence of obesity.

Based on their olfactory function, individuals can be classified as normosmic, hyposmic or anosmic depending on whether they show a normal, reduced or absent ability to perceive odors. This aspect becomes even more complex when considering that human perception of odors differs greatly between individuals, especially in terms of intensity and perceived pleasantness. The causes of this individual variability are multiple and can be traced back to personal experience, environmental factors and genetics. One of the factors that mainly affects the olfactory function is the natural aging process and the progressive sensory deterioration with age. Although it is commonly accepted that women perform better than men in their olfactory abilities, some studies on a large number of individuals report that there are no sex-related differences in olfactory function of individuals. Finally, olfactory deficits are associated with numerous chronic diseases, such as cancer, neurodegenerative diseases, depression, autoimmune/inflammatory diseases, diabetes and obesity, and several studies have reported a relationship between olfactory function, body weight and metabolic state.

The present research project aims to study, through molecular biology and psychophysical techniques, the physiological (age, sex, nutritional status, etc.), genetic (OBPs polymorphisms, Kv1.3 channels, etc.) and environmental (lifestyle and eating habits) factors underlying the variability of olfactory function both in healthy individuals and in particular

physio-pathological conditions (overweight and obesity, inflammatory and/or autoimmune diseases, etc.).

Ornella Sassu

Tema di ricerca: *"Mapping the molecular landscape of thyroid cancer via multi-omics integration."*

Abstract: Thyroid carcinoma (TC) is the most common endocrine malignancy, affecting 0.2-1.5% of individuals worldwide, and its incidence has increased 300% over the past 3 decades. The increased detection of TC has been attributed to increased imaging and diagnostic scrutiny. Thyroid cancer is primarily categorized into several subtypes based on the degree of differentiation and the type of thyroid cells from which they arise. Well-differentiated TC includes papillary thyroid cancer (PTC), accounting for approximately 80-90% of all thyroid cancer cases, followed by Follicular Thyroid Cancer (FTC). Although both of which are relatively responsive to traditional therapy and have a good survival rate, 5–20% carry a significant risk of relapse and mortality. Undifferentiated TC includes anaplastic thyroid cancer (ATC), accounting for 1–2% of all TC cases, which is one of the most aggressive malignancies, characterized by rapid growth and resistance to treatment. Fine needle aspiration cytology (FNAC) is the most accurate, rapid, safe, and cost-effective test for the evaluation of thyroid nodules, with high specificity and sensitivity. Nevertheless, FNAC is particularly unreliable in differentiating between benign and malignant nodules that fall under the category of indeterminate thyroid nodules (class III and class IV according to Bethesda Classification). In fact, in these cases, the expected malignancy rates are 5–15%and 15–30%, respectively. Over the last few decades, many studies have highlighted genetic alterations involved in thyroid carcinoma tumorigenesis, contributing to the development of molecular tests that have improved the estimation of tumor malignancy probability, although still unsatisfactorily. In this context, an increasing number of studies are highlighting the potential of omics technologies in thyroid cancer (TC), indicating their value in identifying biomarkers and in improving both diagnostic and prognostic assessments of various pathophysiological conditions.

Based on this background, this proposal aims to identify novel biological markers of TC able to predict the risk of thyroid nodules malignancy by combining genomic, proteomic and metabolomic profiles adopting a comprehensive multiomic approach. These markers could support clinical decision-making—especially in selecting patients for surgery—and contribute to a deeper understanding of thyroid tumor
biology. Ultimately, this approach may lead to the identification of new therapeutic targets for aggressive or treatment-resistant forms of TC.

XXXIX ciclo (A.A. 2023/24)

Monica Cabboi

Tema di ricerca: *Nutritional impact of gut-targeted supplementation on Metabolic Flexibility in healthy adults.*

Abstract: The gut microbiota is a complex ecosystem composed of bacteria, viruses, and fungal communities that inhabit the human intestine. The composition of this ecosystem can be influenced by various factors, including diet, dietary patterns, and nutrients.

The human gut microbiota plays a central role in regulating metabolic processes, and its modulation through diet and microbiome-targeted supplementation has emerged as a promising strategy to promote health.

Probiotic and postbiotic supplements have gained increasing attention as nutritional strategies to support metabolic health through gut microbiota modulation. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host, primarily by modulating the gut microbiota and enhancing the production of beneficial metabolites such as short-chain fatty acids (SCFAs), including butyrate. Postbiotics, on the other hand, refer to non-viable microbial products or metabolic byproducts, such as SCFAs, that exert biological activity in the host. Among these metabolites, butyrate, in particular, has been associated with favorable metabolic effects, including enhanced lipid oxidation, improved mitochondrial function, attenuation of inflammation, and modulation of gut-brain signaling pathways.

Collectively, these mechanisms suggest that targeted supplementation with probiotics and postbiotics may represent a promising approach to optimize energy metabolism and support overall metabolic flexibility. Metabolic flexibility refers to the ability to efficiently switch between energy substrates in response to physiological demands and its increasingly recognized as a key determinant of metabolic health.

This study project aims to investigate the nutritional impact of probiotics and postbiotics, particularly butyric acid, on metabolic flexibility in different groups of healthy human adults. The research explores both acute and chronic supplementation strategies, including interventions with postbiotic and probiotic formulations. It aims to assess the impact of supplementation on key metabolic parameters, including host-derived SCFAs, and other bioactive lipid-derived molecules such as ketone bodies, endocannabinoid, and N-acylethanolamine. Additional outcomes include the expression of metabolic genes such as PPAR-α and FGF21, as well as body composition. Metabolic flexibility is evaluated through indirect calorimetry and exercise testing, while anthropometric parameters are measured using bioimpedance. Fecal and plasma samples undergo targeted metabolomic analysis, including lipidomics, via UHPLC-MS/MS, HPLC, and GC techniques.

Francesca Floris

Tema di ricerca: *Study of circulating microRNAs as therapeutic targets for the control of iron accumulation in subjects with transfusion-dependent β-thalassemia*

Abstract: β-thalassemia is a range of hereditary diseases caused by mutation in the β globin gene, leading to heavy anaemia and ineffective erythropoiesis. Iron overload is am serious complication for both transfusion-dependent and non-transfusion-dependent thalassemia. The major mechanisms linked to iron overload are red blood cell transfusion and increased intestinal absorption, respectively. Iron metabolism is finely tuned by transcription factors, iron responsive elements, and miRNAs. Indeed, there is the rationale to investigate miRNA dysregulation in both transfusion-dependent and non-transfusion-dependent thalassemia characterized by an iron overload condition.

Ilenia Iamundo De Cumis

Tema di ricerca: *The role of molecular biomarkers as potential predictors of treatment response in patients with locally advanced rectal cancer undergoing chemotherapy and radiotherapy*

Abstract: The project includes a retrospective arm, currently underway, which will focus on a sample of 33 patients treated between 2021 and 2023. The project activities carried out during the first year consisted in reviewing patients' medical records, analyzing demographic data, tumor clinical and instrumental characteristics, and specifically, analyzing staging exams performed by the patients (endoscopic, biopsy, and radiological). Additionally, we reviewed radiotherapy records to evaluate the doses received by the target area and surrounding organs at risk. We also analyzed side effects related to the therapy, any resulting treatment interruptions, and how these may have impacted the therapeutic response. We are also in the process of retrieving biopsy samples (both pre- and post-surgical) from this group of patients to measure miRNAs' expression profile to potentially identify biomarkers of response to TNT. The miRNAs differentially expressed between response groups will then be tested as potential predictors in the prospective part of the project. In this regard, we will soon begin selecting patients eligible for TNT treatment. On the diagnostic biopsy sample for carcinoma, baseline and post intervention miRNAs expression levels will be evaluated to explore their changes across the timepoints.

I am also working on parallel project that aims to identify a panel composed by molecular biomarkers and global longitudinal strain (a specific echocardiographic test that assesses left ventricular function) as potential early predictor of post-treatment cardiac toxicity in patients with mediastinal lymphoma. So far, we have selected and enrolled 10 patients; the protocol consists of molecular and instrumental evaluations at baseline (T0), 48 hours after chemotherapy, 48 hours after radiotherapy, and then every 3 months. The panel of peripheral blood molecular biomarkers include B-type natriuretic peptide (BNP), high-sensitivity troponin (T-HS), and cardiac-specific micro-RNAs (miRNA) (miR-1, miR-133, miR-145, miR-208, miR-499), which will be measured using real-time PCR.

Instrumental evaluations include cardiac computed tomography angiography (CCTA) and cardiac magnetic resonance imaging (MRI) at T0, and echocardiograms (ETG) with 2-dimensional global longitudinal strain (2D-GLS) at each time point. So far, we have observed that changes in GLS and increase in high-sensitivity troponin levels are correlated with early subclinical cardiac alterations detected by echocardiography. This has enabled the identification of a group of patients who may benefit from an early therapeutic approach. We are currently planning the RT-PCR analysis of cardiac miRNAs to assess whether these early cardiac alterations are also correlated with changes in the selected miRNAs"

Ylenia Lai

Tema di ricerca: *The role of proinflammatory environment and innervation in the autologous tissue flaps after ischemia/reperfusion in reconstructive microsurgery*

Abstract: My PhD project is focused on the role of the proinflammatory environment and innervation in the autologous tissue flaps after ischemia/reperfusion in reconstructive microsurgery and aims to characterize the structural and molecular substrates involved in ischemia/reperfusion (I/R) induced tissue damage in human skin flaps treated pre-transplant according to the traditional warm ischemia/reperfusion (I/R) method compared to pre-transplant treatment with a perfusion-preserving solution to prevent I/R damage under cold ischemia conditions. Autologous transplantations are generally the most used due to their higher flap success rate, lower rejection-induced flap, loss of only 1%, and absence of a need for immunosuppressor therapy. This type of transplant is frequently used in breast reconstruction, where skin flaps are taken either from the inner thigh or the abdominal area. The scientific problem is based on the known effects of I/R damage arising due to the absence of perfusion; reperfusion, which would itself be a desirable event, causes oxidative stress in the tissue, due to the re-establishment of circulation, and activates a physiopathological tissue reaction which is mainly based on the activation of a series of oxidative and proinflammatory events.

Micaela Rita Naitza

Tema di ricerca: *Molecular characterization of patients affected by refractory reumatoid artritis*

Abstract: Background Remission is an important goal of therapy in rheumatoid arthritis (RA), but there is a quote of patients that not respond to multiple therapies (>3 bioDMARDs switch), named “refractory”. Nowadays, data on molecular players of clinical remission and effective disease inactivation are scarce: patient’s gene expression profiling is a useful approach to elucidate the pathogenic mechanisms of diseases, and differential gene expression analysis between diverse disease conditions produces gene signatures characteristic of the state or disease being studied.

Objectives The main aim of this project is to molecularly characterize refractory AR patients, comparing first the transcriptional profiles of patients with clinically active versus inactive RA, and versus healthy controls (HCs). The derived gene-candidate list responsible for remission achievement, will be tested on a cohort of refractory AR.

Methods From a cohort of around 1000 patients affected by RA according to ACR-EULAR 2010 criteria, were first selected 20 patients with active disease state (without biologic DMARDs treatment ongoing) (group A) and 20 patients with >1-year remission induced by TNFα antagonism (Etanercept) (group R), as assessed by DAS28(PCR) scores, and from 20 HCs matching for mean age and gender ratio. Both RA groups were not on corticosteroid treatment. Each condition has been profiled using whole blood-derived RNAs pools in biological duplicates by distinct Affymetrix Human GeneChip HTA 2.0. Data analysis was performed using the commercial software Partek Genomics Suite, V 6.6. To identify a transcript as differentially expressed, a value of fold change 1.5 and p-value 0.05 has been set. For all lists of differentially expressed transcripts, the Gene Set Enrichment Analysis by Gene Ontology (GO) was carried out, to identify how molecular functions, cellular components or biological processes occurs more frequently than expected in a reference list of transcripts. The list of transcripts belonging the comparison R vs HC were selected for the validation phase of this project, that will be performed on a cohort of refractory AR (paired with R and A groups), by single-gene quantification (rqPCR technique), starting from a list of

candidate coding DEGs (differentially expressed genes) that will be selected on their physio-pathologic features and literature. Results The Venn diagram shows all comparative groups (A vs R, A vs HC, R vs HC) with their relative number of transcripts differentially expressed, and the relationship between sets (fig1, panel A). Using the list of transcripts differentially expressed in all comparisons, a heat map with the hierarchical clustering was carried out to view the intra-condition expression profile. Here, were identified (arbitrarily) 4 clusters of transcripts with analogous transcriptional profile and to each of them a color code has been assigned (Heatmap in Fig1, panel B). The Gene Set Enrichment Analysis by Gene Ontology (GO) showed that "immune system processes" category occurs more frequently than expected in all list of mRNAs (fig 2 A and B). The validation phase is still ongoing, together with the patient's recruitment. Conclusion Considering the amount of differentially expressed transcripts and their hierarchical clustering, is evident that the drug-induced remission (R) is more similar with HCs condition, while active disease state (A) has a different profile. However, a list of 147 transcripts were highlighted in the R condition that differ from the healthy state. The Gene Set Enrichment Analysis Score showed that mRNA transcripts dysregulated in the R condition are involved in several biological processes regarding the immune system, response to stimulus, biological regulation, locomotion and others, maybe reflecting the good response to TNFalpha antagonism. The next step will be to validate, by Real Time PCR in a large cohort of refractory vs remission patients, the most interesting dysregulated genes covering biological functions eventually sustaining – or not – the remission of disease.

Ali Qaisar

Tema di ricerca: *Targets of TDP-43 (TBPH) regulation*

Abstract: I am working now on the project entitle ''Targets of TDP-43 (TBPH) regulation''. TBPH is a DNA/RNA binding protein member of hnRNP family implicated inneurodegeneration (ALS and FTD). We used three types of TBPH Drosophila model flies (W1118, TBPHΔ23 ,TBPHΔ142). After RNA-seq, we identified three genes involved in splicing and four genes related to epigenetics in the knockdown, compared to the wild type (w111).

We performed Relative expression analysis using Molecular techniques like PCR. We also used Climbing assay for Behavioural analysis.

XXXVIII ciclo (A.A. 2022/23)

Eleonora Grecu

Tema di ricerca: *Assembly of organoids from Alzheimer's disease patients: an integrated 3D model to decrypt the complexity of dementia-related alterations in human brain.*

Abstract: During the second year of the PhD Program in Molecular and Translational Medicine, I carried out my research activity that is still in progress at the Neural Aging Laboratory, Dpt of Neurosciences, University of California San Diego (UCSD), under the supervision of Prof. Jerome Mertens. Driven by the original protocol aimed building a solid 3D in vitro model of Alzheimer's Disease (AD) in shape of human brain organoids, I invested this part of my PhD in developing two different protocols. First, I completed the optimization of the protocol to obtain forebrain organoid (FO) starting from blood- derived human induced pluripotent stem cells (hIPSCs) and I built 44 days old "mini brains" by the following steps: after the generation of the hIPSCs from control healthy samples (Fig. 1, A), I detached them with Collagenase IV and plated them in ultra-low attachment (ULA) dishes for the formation of the Embryoid bodies (EBs), as precursors of the FOs. I cultured them for 1 week, switching the media to the neural induction (NI) one to promote the neuroectodermal differentiation. This fundamental stage can be achieved only and if EBs display smooth edges and a bright outside layer and, therefore, guaranteeing the progression of the protocol (Lancaster et al, 2014; Qian et al, 2016) (Fig. 1, B). After the selection of the most suitable EBs, I embedded them into geltrex at day 7 (Fig. 1, C) and switched to the FO differentiation media at day 14 to allow their 3D expansion. At day 20, all the budding FOs that exhibited rosette-like structures resembling petal flowers were cleared out from geltrex to prevent them from becoming cystic (Fig. 1, D). I fed them until day 44, when they were showing a more extensive (Graph 1), weightier and deeply twisted structure with a necrotic core, due to the lack of oxygen and nutrients distribution into the inner layers during the growth (Fig. 1, E).

The second protocol, already developed in the host lab, introduced me into the field of transdifferentiation by the direct conversion of fibroblast into Induced Neurons (iNs), a 2D model that, bypassing an intermediate stem cell-like or embryo- like pluripotent state, can keep a senescence profile in ricapitulating AD (ZhouYang et al, 2021). INs are so extremely versatile that could be also exploited for gaining 3D structures, as cryogel-microcarriers (a pilot experiment).

The achievement of iNs from both AD and control samples required these steps: fibroblasts in culture needed TFM media to reach the 70-80% of confluency before undergoing transduction for the conversion. In the meanwhile, I became familiar with producing the UNA-lentivirus, a "all-in-one" vector system with Ngn2 and Ascl1 linked via a 2A peptide sequence (Herdy J et al, 2019). When the fibroblasts reached their maturation, I transduced them, waiting 72 hours before switching to TFM-P media in order to sort only the cells competent to the conversion. After having selected and amplified them, until reaching 100% of confluency, I started conversion with NK4 media, a process that takes only 21 days to give completely mature neurons already expressing MAP2, TUJ1 and NEUN markers, regardless of sample type. Starting by the assumption that there is not a unique universal model able to figure out the human features of AD, it is well accepted that iNs represents a "fast" model that preserves aging, but it is not able to recapitulate the complexity of the brain (Heidari et al, 2021), as hIPSCs-derived FOs do, although they could epigenetically undergo a rejuvenation phenomenon (Mertens et al, 2018, Meisnerr et al, 2008). Since it is renowned that blood cells are less prone to gene arrangements during reprogramming (Staerk et al, 2010), my future plan is to try to set up a protocol to directly convert them into iNs to have both 2D and 3D models from AD and control sources.

Finally, I aim to perform a comparison between those models got from both fibroblast and blood cells to disclose possible differences and potential usefulness in terms of phenotypic assessment of cellular types as well as of aging and inflammation biomarkers expression in AD.

Francesco Lai

Tema di ricerca: C*linical and molecular characterization of inherited retinal degeneration (Inherited Retinal Dystrophies, IRDs)*

Abstract: IRDs are a diverse group of neurodegenerative eye disorders, primarily marked by progressive retinal degeneration that often results in significant visual impairment and blindness. These conditions exhibit considerable genetic and phenotypic variability, with differences in age of onset, disease progression, and potential associations with systemic signs and symptoms. To date, mutations in over 250 genes have been linked to IRDs. Despite advances in genetic testing, the diagnostic yield remains between 50% and 80%, leaving many patients without a confirmed diagnosis.

My project focuses on two main objectives.

The first is to investigate the genetic landscape of inherited retinal diseases in Sardinian patients and create a clinical and genetic database for these conditions. This involves comprehensive clinical evaluations, including eye examinations, family pedigree analysis, retinal imaging, electrophysiological studies, and tests to identify any extraocular signs and symptoms. Blood samples are then collected from each patient to perform genetic testing, primarily using Whole Exome Sequencing (WES) and SNP-array in order to investigate the possible presence of Copy Number Variants (CNVs).

The second objective is to address undiagnosed cases by validating the potential association between candidate gene variants and IRDs, and determining the pathogenicity of novel or ultra-rare variants in known genes currently classified as variants of uncertain significance (VUS). To assess the pathogenicity of these variants, in silico models and computational analysis are usually employed to study their impact on protein structures, and in vitro functional studies.

As to the second task, in these months I focused on the novel homozygous missense variant c.346G>A (p.Ala116Thr) in the exon 3 of the CTSD gene detected in a 68-year-old Sardinian patient with adult-onset retinal degeneration and neurological involvement. Cathepsin D, encoded by the CTSD gene, is a ubiquitously expressed lysosomal protease that is involved in proteolytic degradation, cell invasion, and apoptosis. Biallelic pathogenic variants in CTSD are associated with CLN10 disease, a congenital form of NCL.

It represents the earliest and most severe form of NCL with onset before or around birth. Since our patient's phenotype differs from that usually described in patients with CTSD-related disorders, we performed different in silico and in vitro studies to clarify the pathogenicity of our variant. Western blot analysis and CTSD enzymatic activity on cultured fibroblasts confirm the pathogenicity of our variant, showing reduced levels of mature CTSD, and lower levels of cathepsin-D enzymatic activity. The impact of the mutation on the protein was investigated by molecular dynamics simulations. According to the in-silico results the overall effect of the mutation is an increased rigidity of the protein, which can be the source of a decreased functionality.

Antonio Luigi Manai

Tema di ricerca: *The role of vgf peptides as diagnostic plasmatic biomarkers of Parkinson's disease*

Abstract: The main aim of the project is to evaluate the role of vgf peptides as diagnostic plasmatic biomarkers of Parkinson's disease (PD). In particular, I measured the levels of different vgf derived peptides named N-terminus, GGEE, NAPP, TLQP, AQEE and C-terminus through competitive ELISA assays in blood samples of PD patients subdivided in two cohorts depending on the years of treatment (group 1: 1-5y and group 2: >5y) compared to age-matched controls. The experiments that I performed showed that only one kind of vgf peptides (VGF C-terminus) remain significantly decreased in the cohort of the group 1 , while all the others measured were similar to the controls. Hence, to ensure the specificity of the VGF changes related to PD, the VGF C-terminus peptides were measured by ELISA in plasma samples from patients affected by other diseases including inflammatory (multiple sclerosis, lupus erythematosus), neurological (dystonia) and psychiatric diseases. Additionally, to this, I was also involved in other projects, one of these, aims to quantify the stress related to pregnancy, measuring the cholesterol as well as oxytocin and vasopressin in saliva samples, in women (before and after partum). I was doing the collection of the saliva samples which is still ongoing.

The other project regards the study of the VGF peptides on one animal model obtained with overexpression of human alpha-synuclein through a virus vector that mimics the early PD. Hence, I performed some experiment in Milan, thanks to the collaboration of the Prof. Graziella Cappelletti using a new immunofluorescence method named PLA (proximity-ligation-assay) on brain sections from these animals to investigate the specific localization of vgf peptides in the brain. I have also collaborated in a project regarding the toxic role on the fipronil pesticide in the 6-OHDA rats brain demonstrating that the toxin has the ability to modulate the production of pro-VGF and/or its C-terminal truncated peptides in the nigrostriatal system indicating its intimate interaction with the dopaminergic neurotransmission.

Stefano Mariani

Tema di ricerca: *Possible mechanism of action of a methyltransferases inhibitor in cholangiocarcinoma*

Abstract: Cholangiocarcinoma (CCA) is a very difficult-to-treat cancer. Chemotherapies are little effective and response to immune checkpoint inhibitors is limited. Therefore, new therapeutic strategies need to be identified.

Objective: We characterised the enzyme protein arginine-methyltransferase 5 (PRMT5) as a novel therapeutic target in CCA.

Design: We evaluated the expression of PRMT5, its functional partner MEP50 and methylthioadenosine phosphorylase (MTAP)-an enzyme that modulates the sensitivity of PRMT5 to pharmacological inhibitors-in human CCA tissues. PRMT5-targeting drugs, currently tested in clinical trials for other malignancies, were assessed in human CCA cell lines and organoids, as well as in two immunocompetent CCA mouse models. Transcriptomic, proteomic and functional analyses were performed to explore the underlying antitumoural mechanisms.

Results: PRMT5 and MEP50 proteins were correlatively overexpressed in most CCA tissues. MTAP was absent in 25% of intrahepatic CCA. PRMT5-targeting drugs markedly inhibited CCA cell proliferation, synergising with cisplatin and gemcitabine and hindered the growth

of cholangiocarcinoma organoids. PRMT5 inhibition blunted the expression of oncogenic genes involved in chromatin remodelling and DNA repair, consistently inducing the formation of RNA loops and promoting DNA damage. Treatment with PRMT5-targeting drugs significantly restrained the growth of experimental CCA without adverse effects and concomitantly induced the recruitment of CD4 and CD8 T cells to shrinking tumourous lesions.

Conclusion: PRMT5 and MEP50 are frequently upregulated in human CCA, and PRMT5-targeting drugs have significant antitumoural efficacy in clinically relevant CCA models. Our findings support the evaluation of PRMT5 inhibitors in clinical trials, including their combination with cytotoxic and immune therapies.

Alessia Mascia

Tema di ricerca: *Evaluation of Multiple Myeloma's venetic variants to predict disease's progression or remission*

Abstract: During my PhD program, I collected clinical and experimental data of Multiple Myeloma's patients to evaluate disease's progression or remission according to genomic variants and the presence of primary translocations and chromosomal gains or losses.

First, I started with an immunomagnetic selection of plasma cells (PCs) by CD138+ antibody, with a MACS separator (Miltenyi Biotec), according to the manufacturer's protocol. Afterwards, genomic DNA was extracted by plasmacells with silica-membrane-based nucleic acidkit, then it was quantified byNanodrop and Qubit® 2.0 Fluorometer, to evaluate quality and the certain amount.

In the second time, I proceeded with SNP arrays' experiments to evaluate genomic profiling and genomic alterations frequently involving gains and losses of chromosomes and genes involved in these regions. Then, I did exome's experiments on same samples to identify single nucleotide variants and small insertions and deletions (SNVs and INDELs).

At diagnosis, NGS studies are not routinely performed and FISH is still the main approach to molecular characterization of the cancer cells in MM. This carries the intrinsic limitation of investigating only a handful of CNAs and translocations. However, knowing the complexity

of the MM genome the amount of information FISH can return is limited, and so could be its prognostic value in comparison with other approaches.

I analyzed genomic Pathogenic and Likely pathogenic variants detected in most recurrent genes ofmy Sardinian cohort by informatic tool and I studied the percentage of these.

Later time, I compared the SNP array profile with low-coverage whole genome sequencing (WGS) profileto evaluate whether it can be an optimal technique to detect the same duplications and deletions, and it can show the level of translocation as a rearrangement with a small deletion and a small duplication in two chromosomes involved.

In the last time, I proceeded with plasma separation from total blood samples, from which cell-free DNA will be extracted. cfDNA is sequencied to evaluate genomic variants already detected by genomic DNA with molecular techniques such as SNP array and NGS.

Angela Maria Mereu

Tema di ricerca: *Iron-erythropoiesis status correlation: cytofluorimetric analysis*

Abstract: My research activity focused on immunophenotype (IF) analysis of blood samples of β-thalassemia patients throught the flow cytometer BD FACSLyric.

I researched new antigenic markers to isolate erythroid precursors from peripheral blood and I optimised the experimental protocol to differentiate the various erythroblast populations. I am able to identify and quantify different populations of erythroid precursors and I am also autonomous in a gating strategy for optimizing their cytofluorimetric analysis.

I am working on new protocols for studying the maturation curve of eritroid precursors in patients with transfusion-dependent β-thalassemia.

I acquired molecular biology investigation to detect gene mutations involved in blood cancer (BCR-ABL, FLT3, JACK 2 V617F, CALR, MPL). The main techniques I am working on are PCR, Real Time PCR (RT- PCR), Agarose Gel Electrophoresis and Capillary Electrophoresis (CE). I also performed the Chimerism analysis for monitoring the state of HSCs after allogeneic transplant by the employment of Short Tandem Repeats (STR) coupled with CE. Finally, I am capable of detecting and quantifying interleukins in patients with suspected Graft versus Host Disease (GVHD) using the direct ELISA method.

Debraj Mukhopadhyay

Tema di ricerca: *Evaluation of the role of miRNAs as potential biomarkers of diagnosis and prognosis of Malignant Pleural Mesothelioma (MPM) and Non-Small Cell Lung Cancer (NSCLC)"*

Abstract:

The aim of my PhD project is to evaluate the role of miRNAs as potential biomarkers of diagnosis and prognosis of Malignant Pleural Mesothelioma (MPM) and asbestos related lung cancer (LC).

Discussion: microRNA let_7a_5p have shown significant up-regulation shown diagnostic accuracy (>90%) in differentiating patients with malignant pleural mesothelioma from benign asbestos-related pleural effusion.

On the other hand mir_151a_5p may be used as diagnostic and prognostic biomarkers or even a therapeutic target in cancer studies. The expression levels (concentration) of miRNA in the EBC medium are significantly lower than those in the blood plasma medium. This discrepancy might be due to suboptimal SOPs. Therefore, it is necessary to revise our SOPs and repeat the miRNA lab analysis. This limitation may explain why we are unable to provide reliable results at this stage. The primary limitation of our study is the small sample size. Another possible limitation could be that, these biological samples i.e., miRNAs were the oldest samples and maybe with the time the miRNAs in the EBC samples were decreased. The present pilot study was preliminary to a vast-scale screening as a longitudinal study of workers exposed (formerly and current) to asbestos to explore the feasibility of using the non-invasive EBC sampling to detect the miRNA profile and possible new miRNA signatures as early biomarkers of asbestos-related lung cancers (LC) and malignant pleural mesothelioma (MPM).

Mara Persano

Tema di ricerca: *Prognostic/predictive factors for primary liver cancers*

Abstract: Introduction: The aim of this retrospective proof-of-concept study was to compare different second-line treatments for patients with hepatocellular carcinoma and progressive

disease (PD) after first-line lenvatinib or atezolizumab plus bevacizumab. Materials and methods: A total of 1381 patients had PD at first-line therapy. 917 patients received lenvatinib as first-line treatment, and 464 patients atezolizumab plus bevacizumab as first-line.

Results: 49.6% of PD patients received a second-line therapy without any statistical difference in overall survival (OS) between lenvatinib (20.6months) and atezolizumab plus bevacizumab first-line (15.7months; p = 0.12; hazard ratio [HR]= 0.80). After lenvatinib first-line, there wasn't any statistical difference between second-line therapy subgroups (p = 0.27; sorafenib HR: 1; immunotherapy HR: 0.69; other therapies HR: 0.85).

Patients who underwent trans-arterial chemo-embolization (TACE) had a significative longer OS than patients who received sorafenib (24.7 versus 15.8months, p < 0.01; HR=0.64). After atezolizumab plus bevacizumab first-line, there was a statistical difference between second-line therapy subgroups (p < 0.01; sorafenib HR: 1; lenvatinib HR: 0.50; cabozantinib HR: 1.29; other therapies HR: 0.54). Patients who received lenvatinib (17.0months) and those who underwent TACE (15.9months) had a significative longer OS than patients treated with sorafenib (14.2months; respectively, p = 0.01; HR=0.45, and p < 0.05; HR=0.46).

Conclusion: Approximately half of patients receiving first-line lenvatinib or atezolizumab plus bevacizumab access second-line treatment. Our data suggest that in patients progressed to atezolizumab plus bevacizumab, the systemic therapy able to achieve the longest survival is lenvatinib, while in patients progressed to lenvatinib, the systemic therapy able to achieve the longest survival is immunotherapy.

Giorgia Zedda

Tema di ricerca: *Identification of new diagnostic and therapeutic molecular targets In vivo and in vitro models of tumoral and metabolic diseases*

Abstract: The aim of my project, under the supervision of Prof. Andrea Perra, is to identify new molecular targets for metabolic and cancer diseases through the use of in-vivo and in-vitro models. In this context, hepatocellular carcinoma (HCC), the sixth cause of cancer-related deaths worldwide, represents a serious health problem. At the moment, systemic

therapies offer only modest clinical benefits, therefore new therapeutic strategies and new diagnostic biomarkers for HCC are urgently needed.

Experimental animal models represent an important tool for studying the metabolic alterations underlying the development and progression of HCC. Among preclinical investigations, several studies in experimental animal models of hepatocarcinogenesis have contributed to the characterization of the molecular mechanisms underlying the development and progression of HCC. We first analyzed the metabolic and pathological alterations in rats subjected to a hepatocarcinogenesis model characterized by the administration of a single dose of the carcinogen DENA followed by a CMD diet regime in order to promote the development of HCC (4). Then animals were subjected to T3 treatment, that, as expected (5), resulted in the partially regression of the preneoplastic and neoplastic lesions.

As expected, the results showed that treatment with T3 causes a significant reduction in the number and size of the so called persistent lesions, which are positive for (GSTP) and for the metabolic enzyme glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the oxidative branch of pentose phosphate pathway (PPP), that is highly expressed in several human cancers. Immunohistochemical (IHC) analysis for GSTP, confirm the development of preneoplastic nodules.

After IHC characterization, lesions from treated and untreated rats were laser-microdissected and total RNA was extracted. A total of 22 samples were collected and checked for integrity and quality.

We will perform RNA sequencing and bioinformatics analysis to compare the transcription levels between the two groups. The objective of this analysis is to identify and reconstruct the pathways involved in tumour development.