DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
XXXVIII CICLO

Research Project PI: Prof. Tiziana Alberio

TITLE: The role of Rab proteins in the pathogenesis of Parkinson's disease.

ABSTRACT

Rab proteins are molecular switchers able to pass from an inactive to active form, bound respectively to a GDP or a GTP unit, and from a cytosolic to a membrane localization. They are anchored to different cellular membrane compartments thanks to C-terminal prenylation, and their activity is guided by regulator (GAPs, GEFs and GDIs) and effector molecules. Rab involvement in Parkinson's disease (PD) is well characterized, with a familial form related mutation found in Rab39b and Rab32 and the susceptibility locus for the sporadic form containing RAB29 coding sequence. Additionally, several Rab interactors result to be involved in the pathology. Recent studies highlighted a role of Rab proteins in both canonical and alternative mitophagy. Additionally, Rabs alteration of Rab pathways was previously assessed by our laboratory in PARK2 mutated fibroblasts from PD patients. Therefore, we propose here to deepen the molecular characterization of the connection between Rabs and several PD forms (familial and sporadic) by using both targeted biochemical methods and a proteomics/systems biology approach.

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Research Project PI: Dr. Andreina Baj

TITLE: Role of bacteriophage and commensal bacteria cross-talk in a murine model of Inflammatory Bowel Disease

ABSTRACT

The gut microbiota is fundamental in sustaining host local and systemic homeostasis. Unbalance between commensals and pathogens leads to dysbiosis that may be associated with various disorders, including Inflammatory Bowel Disease (IBD). In IBD, dysbiosis contributes to impairment of the epithelial barrier, immune and neuronal cell function, underlaying epithelial injury, altered secretion, enhanced visceral sensitivity and dysmotility. Normalization of these functions by correcting inflammation-related dysbiosis may represent a promising approach to treat the disease.

Development of high throughput sequencing technologies is evidencing the importance of gut prokaryotic bacteriophages in gut microbiota homeostasis and there are hints suggesting that phage composition (phageoma) is altered in IBD patients. Bacteriophages influence bacterial survival and

diversity, and changes in their presence and amount may impact on bacterial ecology. However, the presence of lytic phages in relation to their host bacteria has not been studied in exhaustive detail in gut microbiota both in healthy conditions and during gastrointestinal (GI) inflammation. Thus, the main objective of the present study is to identify for the first time, by means of high throughput genome sequencing, gut commensal bacterial species and strains, whose increased amount in IBD is pathogenetic, and the relevant phages, resorting to a well-established model of dextran sodium sulfate-colitis in mice. Metagenomic microbiota NGS techniques will be performed in fecal and mucosal colonic samples to identify bacteriophages, the related bacteria and bacteriophages/bacteria ratio associated with the pathological state, possibly leading to identify a Phage Therapy to restore the healthy equilibrium. Standard microbiological techniques will be carried out to obtain enriched bacteriophage cultures. The ability of selected bacteriophage cocktails to modify the presence of bacteria leading to secretory, immune and neuronal functions in DSS-treated animals will be studied after carrying out proper toxicological tests.

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Research Project PI: Prof. Marc Ian Bonapace

TITLE: Prostate cancer: disentangling the relationships within the tumour microenvironment to better model and target tumour progression.

ABSTRACT

Background. Prostate cancer (PCa) is one of the most common tumours in men over 60. At diagnosis, 90% of prostate cancers are confined to the organ. Since it is almost impossible to predict the pathological steps that lead to tumour aggressiveness, patients are often treated with partial or radical prostatectomy and/or anti-androgen therapy. However, one third of the patients will progress to the metastatic stage of the disease for which no effective treatments are available. It is clearly emerging that the type of genetic and epigenetic alterations driving malignant transformation in the prostate epithelial cells can predict much better than histology tumour behaviour and sometimes the response to specific therapies. Recently, tumour-microenvironment interplay has been proposed to play a relevant role in tumour progression towards Castration Resistant PCa (15-18). Notably, many of the above features are acquired through Cancer Associated Fibroblasts (CAF)-induced DNA methylation-dependent epigenetic modifications.

Aims. The overall aim is the identification of molecular determinants of the cross-talk between PCa cells and CAFs, determining PCa onset and progression. These goals will be pursued making use of stromal cultures from PCa samples (normal fibroblasts-NFs, and CAFs), and of 2D cell lines cultures modelling tumour progression via genetic manipulation. These models will be used to

study the relationships between oncogenic mechanisms due to cell-intrinsic (genetic and epigenetic) and extrinsic (microenvironment) processes, involved in prostate tumour onset and progression.

Experimental design. For this purpose, the immortalized human epithelial prostate cell line RWPE-1 has been engineered to generate a 2D model of PCa with a panel of doxycycline-based inducible vectors to mimic:

1. ERG over-expression, a very early genomic event in prostate tumourigenesis affecting almost 50% of all PCa patients. ERG is not oncogenic *per se*, yet it sustains tumour progression when combined with a transformation event such as PTEN dysfunction.
2. ERG over-expression in combination with massive PTEN downregulation.

By RNA-seq and Illumina Infinium CytoSNP-850K BeadChip, we will identify differentially expressed and methylated transcripts required for tumour reprogramming in the 2D model. By quantitative Mass spectrometry (MS) performed on the conditioned media of the induced cells, we will also identify secreted factors governing CAF-epithelial crosstalk and prostate tumorigenesis. To validate the driving role of those identified transcripts and secreted proteins, knock down of the up-regulated or over-expression of the down-regulated transcripts will be performed prior to ERG and PTEN modulation in the 2D model, and the resulting phenotypes analysed. The identified secreted proteins will be tested for tumour promoting phenotypic effects on normal fibroblasts and CAFs isolated from radical prostatectomies in collaboration with the "Molinette" Hospital of the University of Turin.

Financing

The project is financed by PRIN 2017 – Progetti di Interesse Nazionale with 125000€ in three years.

External collaborators

Prof. Valeria Poli, University of Turin, Turin

Prof. Paolo Gontero, University of Turin, Turin

Prof. Andrea Lunardi, University of Trento, Trento

Prof. Licio Collavin, University of Trieste, Trieste

Prof. Marco Gaspari, University of Catanzaro, Catanzaro

The PhD candidate will perform his/her PhD thesis in the lab of General Pathology at the University of Insubria and in collaboration with Prof. Valeria Poli at the University of Turin for the -Omics experiments.

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DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE XXXVIII CICLO

Research Project PI: Prof. Elena Bossi

TITLE: Membrane Transporters in health and disease. Role of substrate, inhibitors, and modulation

ABSTRACT

The membrane transporters belonging to SLC6 and SLC1 families are mainly expressed in neurons and glia and involved in the excitation/inhibition balance regulation. These proteins have vital roles in the control of excitability in any brain circuit. Consequently, the comprehension of the functions and dysfunction of these proteins and the possible modulation is fundamental for understanding their roles in many neurological/neurodegenerative/cognitive disorders.
The aim of the project is to investigate the role of substrate and inhibitors in the transport activity and expression, in the presence or absence of regulatory proteins. The biophysical characterization of neurotransmitter transporters, in particular, GABA (GATs), Glycine (GlyTs), and glutamate transporters (EATTs) is the main goal of this research project. The transporters' activity will be studied by heterologous expression and classical two-electrode voltage clamp, but also immunochemistry and molecular biology will be part of the laboratory work. Moreover, different bioinformatics tools will be used to complete the picture of the structure and function interactions.
If you are interested in this project, please contact the PI (elena.bossi@uninsubria.it).
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DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE XXXVIII CICLO

Research Project PI: Prof. Roberto De Ponti

TITLE: Right and left ventricular imaging and inflammatory biomarkers in cardiovascular and non-cardiovascular diseases

ABSTRACT

Background. It is currently well known that a variety of cardiovascular and non-cardiovascular diseases can affect in different ways the morphology and function of the right and left ventricle, detected both by routine and advanced imaging modalities and can correlate with alterations of different biomarkers. Cardiovascular involvement during non-cardiovascular or systemic diseases is currently a fascinating field for research, which is even more interesting in patient affected by neoplasm receiving potentially cardiotoxic therapies.
Currently, it is also known that apart from cardiomyocytes, in the heart different cardiac cell populations, such as fibroblasts, neurons, endothelial and haemopoietic-derived cells form a

myocardial matrix[1], are present with complex intercellular cross-talks and interactions[2]. All these cells contribute to cardiac homeostasis and preserve cardiac geometry and function[3]. Interestingly, RNA sequencing data show that 5-10% of cardiac cells are leukocytes, most of them are macrophages replenished through local proliferation and monocyte recruitment[4], and whose pleiotropic functions are still partly unknown, although they are deemed crucial modulator of many cardiac functions (electrical conduction, efferocytosis, inflammation, tissue development, remodeling and regeneration), and, therefore, cardiac pathophysiology[5]. Many observations and studies suggest in fact the strong contribution of immune cells to many cardiac pathologies, through the innate and adapted immune response, such as myocarditis and inflammatory cardiomyopathy[6,7], where recent pre-clinical observations, based on animal models [8–10], recognize a new immunological mechanism involving the spleen, as one of the main contributors to chronic inflammation, that lead to myocardial remodeling and disfunction, the so-called inflammatory cardiomyopathy. A similar mechanism has been also observed in ischemic heart failure (HF)[11] as well as in non-ischemic heart failure with preserved (HFpEF) or reduced ejection fraction (HFrEF)[12]. Interestingly both HFrEF (either from an ischemic or non-ischemic cause) and HFpEF are characterized by elevated serum proinflammatory cytokines (IL1, IL6, galactine 3, TNFα, TNFR1, TNFR2), which correlate with HF progression[13].
Left ventricular disfunction and HF are not rare in cancer populations, in fact cardiovascular diseases are the most frequent non-cancer cause of death in these patients[15]. The interrelation between cancer and HF can occur at different levels: they share the same risk factors, they frequently show similar symptoms,[15] and, eventually, some studies even suggest a higher incidence of cancer in HF populations. [17] Cancer and HF seem to have a common hallmark, characterized by inflammation.[16,17]
On the other hand, our understanding on cardiac disfunction due to anticancer drugs is rapidly evolving, thanks to the progress of pre-clinical studies[16]. In this biological scenario, the new immune checkpoint inhibitors (ICIs) drugs have emerged as a front-line therapy for solid tumors, showing impressive results, and getting approved for the treatment of many forms of malignant tumors, such as melanoma, renal cell carcinoma, urothelial carcinoma, head and neck cancer and non-small cell lung cancer (NSCLC)[18,19]. These compounds target the so called-immune checkpoint, a complex of membrane receptors crucial for regulating an immune response, awakening an immune response primarily, but not solely, directed against cancer cells[20]. Their widespread use nevertheless has brought important concerns about mid- and long-term risks of cardiovascular diseases, driven by their cardiotoxic effects, that are nowadays still not well-defined. Certainly, one of the most fearsome cardiac side effect of ICIs is drug-induced myocarditis and ICIs are one of the pharmacological classes with the highest risk of severe myocarditis[21]. As shown in recent clinical trials, early detection of troponine elevation during the first weeks of ICIs therapy, may be a useful tool to identify drug-related cardiotoxicity[19,22]. This is consistent with the crucial role of serum biomarkers in patients receiving potentially cardiotoxic cancer drugs[23], that, combined with the pivotal function of contemporary cardiac imaging such as the advanced echocardiographic assessment [24], might find the new strategies to detect, monitor and treat cancer-therapy cardiotoxicity.

Aim. The aim of this research project is to evaluate serum biomarkers of myocardial damage and or inflammation and correlate with routine and advanced imaging data of the right and left ventricle in patients with cardiovascular and non-cardiovascular diseases leading to heart failure and monitor the modification of these parameters to investigate the mechanisms and the evolution driving to cardiac disfunction.

Methods. For this research patients populations will be accurately selected among cohorts with cardiovascular disease (such as systemic and pulmonary hypertension, inflammatory diseases, cardiomyopathies, etc) and non-cardiovascular diseases (neoplasms undergoing different therapeutic strategies, thyroid disfunction, systemic diseases, etc). A particularly interesting patients setting could be represented by patients affected by cancer receiving potentially cardiotoxic treatments, in whom research can aim at monitoring biomarkers and the morpho-functional aspects of the ventricles during and after therapy, detecting and clarifying the mechanism of the development of ventricular disfunction and heart failure over time, regardless of anticancer treatments.
The research methodology used will include systematic and qualitative reviews and metanalysis of the available evidence, as well as retrospective or prospective single-centre or multicenter data collection and analysis of patient series.

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DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
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Research Project PI: Prof. Daniela Furlan

TITLE: Finding drivers of methylation in endometrial cancers

ABSTRACT

Background

Endometrial cancer (EC) is the most common gynaecological malignancy and fourth most common cancer in the developed countries. Both incidence and mortality rates have been rising in the last decade and are predicted to increase further due to risk factors such as diabetes and obesity.

Currently, clinical and histopathological factors such as stage, histotype, grade, depth of invasion, and lymph vascular space invasion are used to stratify patients into risk groups to guide surgical management, adjuvant therapy and follow-up. However, these clinicopathological variables do not sufficiently predict patient outcomes. Recently the results of next-generation sequencing studies have expanded knowledge of recurrently altered signalling pathways in EC and have laid the foundations for rational design of molecular-based clinical trials. Recently the Cancer Genome Atlas Network (TCGA) has reported a comprehensive genomic and transcriptomic analysis of EC. On the basis of integration of mutation spectra, copy-number aberrations, and microsatellite instability status, ECs were categorised into four genomic classes: 1) *POLE* (ultramutated) tumours characterized by very high mutation rates and hotspot mutations in the exonuclease domain of *POLE*; 2) a microsatellite-unstable (MSI, hypermutated) group of endometrioid tumours characterized by microsatellite instability due to *MLH1* promoter methylation and high mutation rates; 3) copy-number low ECs, comprising microsatellite-stable grade 1 and 2 endometrioid cancers with low mutation rates, characterized by frequent *CTNNB1* mutations; 4) copy-number high (serous-like) tumours, characterized by extensive copy-number alterations, low mutation rates, recurrent *TP53*, *FBXW7* mutations and poor outcome. Although the TCGA genomic characterization of EC has not yet entered in the clinical practice, there is a strong scientific rationale that the identification of the four TCGA subtypes of EC might permit a reclassification of these tumors, which could directly affect treatment decisions and guide clinical trials of targeted therapies.

The second important aspect regards the epigenetic profiles of ECs. DNA methylation is highly dysregulated in cancers displaying aberrant CpG island hypermethylation and long-range blocks of hypomethylation.

Although very few reports have been reported on the epigenetic landscapes of ECs, there is accumulating evidence that DNA methylation changes may contribute to carcinogenesis in the endometrium. Aberrant methylation of tumor suppressor genes is detectable in EC precursor and has been shown to distinguish between benign tissue and cancer. These findings suggest that some methylation markers may have value in EC screening, early detection and prevention. So far, more than 50 hypermethylated tumour suppressor genes have been identified including the most famous genes: *MLH1, PTEN, p16, APC, MGMT, RASSF1, PR and CDH1*. On the other hand, few publications have described hypomethylated oncogenes in endometrial cancer (such as *BMP, CTCFL, PARP1, CASP8*). Genes with aberrant DNA methylation are involved in various biological pathways

such as cell adhesion, cell proliferation, signalling transduction, cell cycle regulation, microtubule stabilization and also apoptosis.

Working hypothesis and translational implications

Specific driver gene mutations are tightly tied to tumor DNA methylation landscapes in a site-specific manner. Well known driver genes associated with CpG island hypermethylation include *BRAF* in colorectal carcinomas and *IDH1* in gliomas.

This aspect has not so far been elucidated in EC and there is a gap in our knowledge about driver gene mutations and pathways causing promoter methylation (CpG island methylator phenotype; CIMP) and how these contribute to the four main categories of EC as defined by the TCGA research network.

In particular, there are no clear drivers of the microsatellite instability and CIMP, which occur in a quarter or more of endometrial carcinomas. Of the commonly mutated genes in EC (including *POLE, POLD, PTEN, PIK3CA, PIK3R1, KRAS, FGFR2, ARID1A, TP53, FBXW7*) *PTEN* has been associated with DNA hypermethylation while *TP53* and *CTNNB1* mutations correlate with DNA hypomethylation. However there is no clear evidence that *PTEN* may be a driver gene of promoter methylation in EC.

We propose that the identification of drivers of methylation in EC may be useful to recognize different clinico-pathological subsets of ECs to guide treatment decisions.

Specific aims

Aim 1: Targeted next generation sequencing (NGS) will be carried out in a consecutive series of 150 sporadic ECs diagnosed at Ospedale di Circolo, ASST Settelaghi in Varese (Italy) in the last three years.

This analysis will include all the commonly mutated genes in ECs that will be evaluated for single nucleotide variants (SNV), for small insertion/deletions (Indel) and for Copy Number Variation (CNV).

The mutation profiles of these tumors will be correlated with MSI status and with the tumor clinico-pathological features.

Aim 2:

Specific DNA methylation patterns will be evaluated in the same cohort of 150 ECs performing both DNA hypomethylation and DNA hypermethylation analyses in order to correlate precise genetic mutations with CpG island/promoter methylation and with DNA hypomethylation profiles.

We will perform these analyses on bisulfite converted tumor DNAs using NGS analysis and we will include both hypermethylated tumour suppressor genes and hypomethylated oncogenes in ECs.

Moreover we will analyse LINE-1 methylation sequences using bisulfite-pyrosequencing to evaluate global levels of DNA methylation in ECs.

Collaborators

Dr. Annabelle Lewis, Department of Life Sciences, Brunel University, London, UK

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DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
XXXVIII CICLO

Research Project PI: Prof. Paolo Grossi

TITLE: Programma combinato di *antimicrobial stewardship* sull'utilizzo dei carbapenemi.

ABSTRACT

Introduzione

Il fenomeno della resistenza agli antibiotici è una delle continue sfide delle malattie infettive. Da più di 10 anni l'Italia è al centro di una vera e propria endemia di batteri resistenti agli antibiotici, situazione ulteriormente peggiorata dalla pandemia COVID-19. Un pattern di resistenza preoccupante che è emerso negli ultimi anni a carico dei batteri Gram negativi fermentanti e non è quello della resistenza ai carbapenemi, molecole che a loro volta erano state un'arma nei confronti di batteri produttori di beta-lattamasi a spettro esteso. Le infezioni causate da batteri resistenti ai carbapenemi colpiscono prevalentemente pazienti ospedalizzati affetti da plurime comorbidità ed è stata dimostrata l'associazione con un'aumento della mortalità, soprattutto nei pazienti immunocompromessi. Sebbene negli ultimi 5 anni siano state sviluppate e commercializzate nuove molecole efficaci nei confronti di tali microorganismi, non sono tardate ad arrivare segnalazioni di resistenza anche a questi nuovi farmaci.

I geni di antibioticoresistenza sono codificati nei genomi batterici e sono in grado di diffondersi orizzontalmente all'interno delle colonie tramite vettori, quali i plasmidi, ma la loro espressione è fortemente stimolata dall'utilizzo degli antibiotici. Per tale motivo l'utilizzo ragionato degli antibiotici attraverso programmi multidisciplinari atti a ridurre la pressione antibiotica sui batteri ha

dimostrato di essere in grado di ridurre l'utilizzo inappropriato di antibiotici ad ampio spettro, di ridurre l'incidenza di batteri multiresistenti (MDR) e, collateralmente, la degenza ospedaliera, quindi i costi a carico del sistema sanitario e, in alcuni casi, anche la mortalità. Tali programmi, racchiusi nella definizione di *antimicrobial stewardship*, sono progetti che coinvolgono diverse figure all'interno dell'ospedale, a partire dall'infettivologo, all'infermiere di rischio clinico, dal farmacista al microbiologo, e intendono promuovere, con diverse modalità, l'uso responsabile degli antibiotici.

A partire da queste premesse, il nostro progetto ha come obiettivo quello di valutare l'impatto di un programma combinato di *antimicrobial stewardship* formato da restrizione prescrittiva e valutazione clinica infettivologica in un ospedale di III livello.

Metodi

Proponiamo uno studio clinico pre-post quasi-sperimentale basato su un programma combinato di antimicrobial stewardship formato da restrizione prescrittiva e valutazione clinica da parte dell'Infettivologo nei reparti che maggiormente risultano prescrittori di tali molecole (terapie intensive, ematologia, medicine).

Verranno inclusi tutti i pazienti a cui verrà prescritto un carbapeneme.

La prescrizione dei carbapenemi sarà permessa come terapia empirica e senza la prescrizione dell'Infettivologo solamente per 72 ore. Oltre tale periodo, sarà necessaria una valutazione infettivologica che verrà richiesta secondo i consueti canali informatici e che valuterà l'eventuale appropriatezza prescrittiva.

In ogni caso la richiesta di carbapenemi da parte dei reparti avverrà tramite dei moduli online di richiesta motivata.

I carbapenemi inclusi nello studio saranno meropenem, imipenem ed ertapenem.

Obiettivi

L'obiettivo primario dello studio sarà quello di descrivere l'impatto del programma di *antimicrobial stewardship* sull'uso e sull'appropriatezza prescrittiva dei carbapenemi facendo un confronto con il periodo antecedente l'avvio del programma.

Gli obiettivi secondari saranno:

- descrizione delle terapie mirate in *descalation* dai carbapenemi

- incidenza di nuove colonizzazioni da MDR

- incidenza di nuove infezioni da MDR

- incidenza di nuove infezioni da Clostridioides difficile

- durata del ricovero

- riammissioni per infezione

- mortalità a 14 e a 30 giorni

Conclusioni

Un programma di restrizione prescrittiva combinato con valutazione clinica che riduca l'uso inappropriato di carbapenemi può limitare l'incidenza di colonizzazioni e infezioni da patogeni MDR, abbassare la durata della degenza e i costi.

DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
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Research Project PI: Prof. Charlotte KILSTRUP-NIELSEN

TITLE: Characterization of the role of CDKL5 in the formation of inhibitory synapses

ABSTRACT

Background: mutations in the X-linked cyclin-dependent kinase-like 5 gene (*CDKL5*) cause CDKL5 deficiency disorder (CDD), a neurodevelopmental pathology characterized by early onset of intractable seizures, severe intellectual disability, autistic traits, and hypotonia. The majority of patients are heterozygous females that either do not express CDKL5 or express hypo-functional variants of CDKL5. CDKL5 functions have been investigated in *Cdkl5*-KO mouse models that recapitulate most features of the human pathology. Such studies converge on a role of CDKL5 in regulating synaptic functions through microtubule dynamics and its control of neuronal receptor expression and composition. Recent unpublished data from our lab show that loss of CDKL5 leads to an altered membrane expression of $GABA_A$-receptors, which represent the main inhibitory receptors of the nervous system. The precise molecular mechanism through which CDKL5 causes aberrant $GABA_A$-receptors expression is still not known but we envisage that a deeper understanding would pave the way for therapeutic drug-based strategies.

Objective.

In this project, which has been financed by the Telethon foundation, the student will make large use of molecular, biochemical, and imaging approaches to study how CDKL5 influences inhibitory synapse formation. Primary cultures of *Cdkl5*-KO neurons and cerebral tissue from KO mice will be used together with cell cultures.

Candidates: Interested candidates should be highly motivated to work in a team and have a background in molecular and cellular biology and be willing to work with rodents.

For further details about the project please contact: c.kilstrup-nielsen@uninsubria.it

DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
XXXVIII CICLO

Research Project PI: Prof. Stefano La Rosa

TITLE: Characterization of genetic and epigenetic profile of well differentiated neuroendocrine tumors with high proliferation (G3-NET).

ABSTRACT

Background: well differentiated neuroendocrine tumors (NETs) are currently classified in different prognostic categories including G1-NET, G2-NET and the recently identified more aggressive G3-NET, which is characterized by a well differentiated morphology and high proliferation (>20 mitoses x $2mm^2$ and > 20% of Ki67 index). G3-NETs have been formally included as a specific clinico-pathologic subtype in the WHO classification of digestive neoplasms only in 2019 [1]. However, G3-NETs have also been described in other organs, such as the lung, where they do not officially represent a specific entity due to the lack of phenotypic and molecular information [2]. Preliminary data obtained in digestive G3-NETs suggest that they are different from neuroendocrine carcinomas (NECs), a peculiar neuroendocrine neoplasm composed of poorly differentiated cells but with similar high proliferative profile (>20 mitoses x $2mm^2$ and > 20% of Ki67 index). However, therapeutic and prognostic information indicates that G3 NETs and NECs are two different entities, at least in the digestive system. In addition, although G3-NETs morphologically resemble G1- or G2-NETs, mechanisms involved in the activation of proliferative mechanisms and biological aggressiveness are not known [3-5].

Recent findings suggest that host immune response seems to have a prognostic and predictive role in neuroendocrine neoplasms, although it is not definitively clarified [6, 7]. For this reason the use of immune checkpoint inhibitors is not still approved for such neoplasms.

Specific aims:

1. to study the molecular characteristic including gene fusion, gene mutation and promoter methylation and gene expression profile of lung and digestive G3-NETs
2. to compare the molecular profile of G3-NETs with that of G1/G2 NETs and NECs of the same systems.
3. to correlate the molecular profile with the immunophenotype
4. to correlate the molecular profile with prognosis and response to currently used therapies
5. to evaluate the inflammatory component of G1-G3-NETs and NECs including lymphocytic characterization, PD-1 and PD-L1 expression, and gene expression analysis using the NanoString nCounter gene expression platform.

Materials and Methods:

Cases of lung and digestive NETs and NECs will be included and analyzed using different technologies: NGS (mutation and gene fusion analysis), MS-MLPA (gene promoter methylation analysis), Nanostring technology (gene expression profile) for the key pathways at the interface of the tumor, tumor micro-environment, and immune response), and immunohistochemistry (protein expression).

Expected results:

The present PhD project will elucidate the pathogenetic mechanisms of G3-NETs development and progression and the possible role of inflammatory tumor component in their biology and response to immune checkpoint inhibitors.

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DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
XXXVIII CICLO

Research Project PI: Dr. Roberto Papait

TITLE: Study the role of histone modifications in the metabolic remodelling of heart during aging

ABSTRACT

Aging is the main risk factor for heart failure (HF) in the elderly as it is often accompanied by cardiac malfunctioning making their heart more susceptible to develop this disease. One of the main causes of age-related cardiac malfunctioning is an “energy deficit” that occurs in heart of the elderly, resulting in loss of metabolic flexibility, decline in fatty acid (FA) oxidation rate, and increase of anaerobic glycolysis in cardiomyocytes (CMs). The molecular mechanisms underlying this metabolic remodeling are largely unknown.

At the basis of correct cardiac function lie precise transcription programs specific for CMs, the de-regulation of which can lead to HF. Over the last few years, the use of ‘omics’ approaches to study the epigenome (e.g., chromatin immunoprecipitation coupled with massively parallel sequencing − ChIP-seq) and the transcriptome (e.g., RNA-sequencing − RNA-seq) has revealed that chemical modifications of histone H3 − namely, acetylation and methylation − determine the transcription programs underlying CM differentiation, maintenance of heart homeostasis in adults, and promotion of the transcriptional changes that cause pathological cardiac hypertrophy. Despite this, and the importance that the regulation of expression of metabolic genes has in defining cell metabolism, the role of histone modifications in age-related cardiac malfunctioning remains largely unknown.

The current proposal aims to investigate the role of histone modifications in the metabolic reprogramming occurring in cardiomyocytes during aging through the combination of high-throughput approaches used for studying the epigenome and traditional methodologies employed for studying cardiovascular disease.

DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
XXXVIII CICLO

Research Project PI: Prof. Massimo Venturini

TITLE: Drugs and molecules to antagonize podotoxins inducing diabetic kidney disease (DKD): functional biomarkers and experimental color doppler ultrasounds (CDU) analysis in a murine model

ABSTRACT

Background

Diabetic kidney disease (DKD) is one of the most serious complications faced by diabetic patients. Despite significant progress and an overall improvement in the treatment of DKD, the development of chronic renal failure is nearly inevitable, and diabetes is rapidly becoming the leading cause of end-stage renal disease. Glomerular podocytes, affixed to the glomerular basal membrane, are the final barrier to urinary protein loss as well as the primary targets of hyperglycemia and chronic inflammation that occur during the early phases of DKD, ultimately resulting in podocytes loss and massive proteinuria. We hypothesize that a pro-inflammatory/hyperglycemic environment such as that observed systemically and locally in patients with diabetes, may promote an abnormal increase in the circulation of some circulating proteins, which further targets kidney glomeruli in the onset/progression of DKD. Indeed, recent studies have suggested that a kidney inflammatory signature exists in patients who develop severe and rapid DKD and may play a role in mediating kidney damage. Within the kidney, these factors may favor podocytes death, thus acting as a novel *Podotoxins*. We further hypothesize that blocking this signaling, may reduce the extent of podocytopathy and the progression of kidney injury, thereby providing a new therapeutic tool for DKD.

Aim

To demonstrate our hypothesis, we will employ in vitro and vivo experiments and we will take advantage of some tools, such as in vivo imaging, which is very effective in visualizing kidney delivery of drugs and molecules and track labelled drugs and molecules within the kidney structure. This will allow us to unveil a novel signaling able to directly damage kidney glomeruli during DKD and identify the mechanism of action in vivo. Finally, this will also represent an opportunity to develop new therapeutics aimed at targeting the podotoxins identified and to explore their delivery into the kidney.

Methods

In order to abrogate the deleterious effects of podotoxin signaling we will develop novel tools, including but not only, monoclonal antibodies, small molecules and fusion protein based on the extra cellular domain of the putative podotoxin receptor.
Pathological (i.e.; synaptopodin staining, etc) and functional biomarkers (i.e.; serum creatinine, urinary albumin, total urinary volume) will be assessed. Experimental color-Doppler ultrasound (CDU) with high-frequency probes (40-70 MHz) will be used to test the deleterious effect of podotoxin and subsequently the beneficial effect of monoclonal antibodies (and other molecules/drugs). CDU will be used by a single operator experienced in CDU on a murine model. Quantitative measurements (arterial resistive index) will be performed to test the kidney status. Biomarkers and CDU findings will be related.

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DOTTORATO DI MEDICINA SPERIMENTALE E TRASLAZIONALE
XXXVIII CICLO

Research Project PI: Prof. Dina Visca

TITLE: Severe asthma and biological treatment: effect on inflammation and cardiovascular diseases

ABSTRACT

Severe asthma afflicts 5 to 10 percent of the asthma population, but drives the majority of the morbidity and costs of the disease. Severe asthma patients are subjects who require high-dose inhaled or near continuous oral glucocorticoid treatment to maintain asthma control or who never achieve control despite that treatment. Approximately 70 percent of severe asthma is associated with persistent elevation in markers of Type 2 inflammation (blood eosinophils, fraction of exhaled nitric oxide [FeNO] and sputum eosinophils). Some of these patients may be candidates for one of the biologic agents, such as anti-immunoglobulin E (anti-IgE) therapy, anti-interleukin-5 (anti-IL-5), anti-IL-5R, anti-IL-4R depending on clinical criteria and biomerkers of Type-2 inflammation. The European Respiratory Society/American Thoracic Society guidelines suggest that treatment of severe asthma be guided by clinical criteria and biomarkers such as blood, sputum eosinophil counts, or fraction of exhaled nitric oxide (FE_{NO}). Peripheral blood eosinophils appear to be good predictors of

response to Type-2 targeted therapy. However, it is likely that additional Type-2 biomarkers will be identified, which subdefine Type-2 asthma even further, and which will eventually better guide therapy than the current blood eosinophils and FeNO. Response to treatment may also depend on the presence of comorbidities. Common comorbid conditions are often disproportionately present in severe asthma. Optimal care of patients with asthma requires the recognition and treatment of these comorbid conditions. Population-based studies have reported that asthmatic adult patients are more prone to have cardiovascular diseases than non asthmatic patients (i.e. hypertension seems to increase with asthma severity: 23%, 29% and 36% for mild, moderate and severe asthma respectively). The relationship between cardiovascular diseases and asthma may be synergistic and due to common mechanisms such as vascular remodeling and endothelial abnormalities (e.g. abnormal contraction and proliferation of smooth muscle cells). Both pulmonary and cardiovascular regulation are under autonomic control and there are many interdependent parts, which may explain the relationship between asthma and hypertension. However, this relationship still needs to be further investigated, particularly the effect of biological treatment for severe asthma on inflammation and cardiovascular disease. Cardiovascular comorbidities frequently associate with worse asthma control and greater risks of exacerbation and posing challenges for its optimal management. A multidisciplinary approach that addresses these comorbidities may result in better clinical asthma outcomes.