PhD Course in Computer Sciences - XXXVII cycle, a.y. 2021/2022

n.1 scholarship funded by Ospedale San Raffele S.r.l. linked to the project: "Harmonizing genetic and phenotypic trajectories in single cell genomics"

Abstract

Recent technological advances allowed the possibility to study the molecular profiles of thousands of single cell at the same time. While the most popular approach is based on transcriptome profiling, there is a profusion of methods to analyze other molecules and their properties, expanding possibilities to genetic and epigenetic profiles. We at COSR developed a strategy, scGET-seq, which is able to profile the genome and the epigenome in parallel on the same cells. This method opens new possibilities in studying complex biological systems, such as cancer. It is widely accepted that cancer evolution is inherently linked to, and can be studied by, the mutational profile of cancerous cells. Recent evidences show that the phenotypic properties, encoded by epigenetics, play an important role in tumor emergence and evolution. The history of a tumor can be then drawn from two different points of view possibly without consensus. The project aims at the development of a computational strategy to harmonize two apparently incompatible trajectories. The key steps will be 1) the implementation of a reliable approach to spot somatic mutations in shallow coverage DNA sequencing; 2) the formalization of a method to extract probabilistic transition kernels from the genetic and the epigenetic modalities; 3) the formulation of a generalized model to unify the trajectory inferences.

PhD Course in Translational and Molecular Medicine - XXXVII cycle, a.y. 2021/2022

n.1 scholarship funded by Ospedale San Raffele S.r.l. linked to the project:” Defining the immune landscape of colorectal cancer metastases in the liver”

Abstract

The project is part of the recently funded AIRC5x1000 Institutional program entitled “Advanced immune gene and cell therapies for liver metastases”, which integrates 4 clinical units and 13 independent research groups located within the San Raffaele campus, leveraging complementary expertise in surgery, oncology, immunology, immunotherapy, cell and gene therapy, and imaging to find new immuno-gene therapy approaches for the hepatic metastases (MTS) from colorectal (CRC) and pancreatic adenocarcinoma (PDAC) (https://research.hsr.it/en/index.html). The major unmet clinical need of these diseases is the poor response to the current therapies of their hepatic metastases. Adoptive cell therapy (ACT) with tumor-redirected T cells, whether by expressing anti-tumor CARs or TCRs, holds promise for the control of metastatic cancers. The efficacy of ACT critically depends on immunosuppressive cues derived from the tumor microenvironment, which must be counteracted by the transferred T cell populations. However, the cellular and molecular suppressive pathways active in the hepatic metastatic microenvironment are essentially unknown and must be defined. Hence, the project aims at unraveling at high resolution the hepatic metastatic ecosystem, focusing on CRC, and define possible effector or suppressive pathways implicated in the local immune surveillance. Specifically, we want to verify the hypothesis that the pre-surgical chemotherapy applied to CRC MTS may induce beneficial effects and potentially targetable changes in the immune profile, via activation of de-novo immuneresponses and/or repolarization of tumor-promoting immune inflammation. To verify the hypothesis, the proposed project will integrate state-of-the-art high dimensional flow cytometry, WES and RNA-seq and multiplexing immuno-histology of primary tumor specimens, and matched peripheral blood, of patients with liver CRC MTS operated at OSR (4-8 patients per month). Distinct infiltrating effector and regulatory immune
cells will be investigated by polychromatic high dimensional 28 color flow cytometry panels (BD Symphony) from single cell suspensions of surgically removed CRC metastatic lesions, and paired autologous PBMCs. The spatial immunecontexture of the metastatic lesions will be reconstructed by multiplexing immuno-histology analysis. Computational analysis and integration of all data will be done in collaboration with expert bioinformaticians. The results obtained in patients will be mechanistically validated in realistic animal model of metastasis ongoing in the lab. The information gathered by multidimensional approach will provide novel knowledge on the crosstalk between cancer and host, and will be instrumental to shape the design of the novel gene and cell therapy strategies proposed by the Program.

Skills to be acquired by the student:
The student will be fully involved in an exciting and ambitious program project embracing clinical and fundamental research, and will have access to cutting-edge flow cytometry, imaging and omics facilities provided by the San Raffaele Scientific Institute. The experimental plan of the project offers a very comprehensive training, which includes: handling of primary clinical samples; hands-on expertise in polychromatic high dimensional flow cytometry, cellular immunological assays, histological multiplexing to provide spatial definition of flowcytometry data, all of which already established and running in the lab, in addition to overall organization and managing skills of complex project involving prospective clinical samples, and single cell computational approaches to analyse data from flow cytometry. The Project program has also activated a continuous flow of surgical samples with the OSR Hepatic surgery Unit and Tissue Biobank worth 60-70 clinically and molecularly (WES and RNA-seq) annotated cases per year, ensuring the progression of the study and the adequate sample dimension for statistics. Furthermore, this project will run in close association with activities by other groups in the AIRC5x1000 Program aimed at T cell engineering and gene editing for optimized ACT of colorectal cancer metastases, in response to the defined hepatic immune landscape. This will generate a rather stimulating environment to foster the student’s training and scientific growth.

n.1 scholarship funded by Department of Medicine and Surgery linked to research project:” Decellularized ovarian cancer matrix as bioactive microenvironment for in vitro 3D cancer research model”

Abstract

BACKGROUND
Ovarian cancer (OC) is the most lethal gynecologic cancer. 5,200 new cases of ovarian cancer are diagnosed each year in Italy, resulting in more than 3,600 deaths. OC is usually diagnosed at an advanced stage and the absence of effective treatments is cause of high risk of relapse (80% in high grade tumors). In the field of drug discovery, the preclinical evaluation of drug cytotoxicity, efficacy, and efficiency is a critical step. Organoids, provide powerful tools to model human diseases at the same time, this kind of technology is still too close to the vision that associates the cancer phenomenon to a cellular-only disease. A plethora of research works demonstrate that the microenvironment is not only architecture capable of supporting the tissue but also a complex network of molecules, which contribute in a fundamental way to the disease evolution. Tissue extracellular matrix (ECM) is a physiologically functional tissue element, responsible for cell adhesion, cell–cell communication, and cell proliferation. Every organ has a distinctive ECM composition to serve particular tissue-specific purposes and functions during tissue development and disease progression. Given the increasingly prominent role that ECM has achieved, many studies have moved to address cancer not only as a disease of uncontrolled cell proliferation but also of dysregulation of the tissue-specific microenvironment.

PURPOSES OF THE STUDY
The purposes of this study are:
To develop a preclinical model for studying the effect of the interaction between tumour and stroma cells and matrix on the evolution of the diseases.

To standardize and characterize a decellularization protocol for healthy ovarian tissue and ovarian cancer tissue counterpart, able to maintain native structural microenvironment;

To develop ovarian cancer organoid (PDOs) cultures with the aim of a better prediction of treatment response.

n.1 scholarship funded by Department of Medicine and Surgery linked to research project:” Investigation of the co-mutational landscape of ALK+ tumors"

**Abstract**

Despite recent advances in targeted therapies, long-term prognosis is still unsatisfactory for a significant fraction of cancer patients. A deeper understanding of the disease at personalized level is needed to improve treatment outcome. Next-generation sequencing (NGS) analyses offer the opportunity to query the landscape of genetic variation in patients and to correlate it to clinical data. This information can be exploited for patients' stratification and for therapeutic purposes. ALK-positive (ALK+) tumors show very good responses to ALK inhibition, yet one third of ALK+ lymphoma and all ALK+ lung cancer patients relapse. Currently, there is no pre-treatment feature in these patients that predicts duration of response. We propose that genetic markers could be explored as response predictors. Our hypothesis is that mapping the full spectrum of genetic lesions present in a tumor may provide useful predictive information on the outcome of targeted treatments. In addition, the identification of co-targets may bring new opportunities to prevent or treat drug resistance. NGS also offers the possibility to monitor molecular response and relapse, and design proper therapies for recurrent disease. We propose to comprehensively analyse the mutational landscape in ALK+ ALCL and NSCLC samples by sequencing: (1) the whole exome; (2) a panel of selected genes at high depth; (3) the whole transcriptome at single-cell level; (4) the circulating tumor DNA. We aim to develop a molecular signature classifier to stratify patients for risk of failing TKI therapy, to identify novel prognostic markers and to characterize the mechanisms of resistance. We plan to apply these findings to the development of combinatorial therapies to treat refractory disease or prevent the rise of TKI resistance in ALK+ cancers. We expect to identify and characterize the somatic events that may co-exist, along with the primary fusion oncogene, in ALK+ tumors, thus influencing TKI treatment outcome. Specific genetic signatures will be correlated to the response to TKI therapy and used to design preventive drug combinations.

PhD Course in Neurosciences - XXXVII cycle, a.y. 2021/2022

n.1 scholarship funded by Zambon S.p.A. linked to research project:” Identification of biological markers, pathogenic mechanisms and therapeutic approaches in Parkinson’s disease"

**Abstract**

From Parkinson’s disease patients. Furthermore, new possible biomarkers of autophagic dysfunctions Parkinson disease (PD), the second most common neurodegenerative disease after Alzheimer’s disease, is characterized by an extensive and progressive loss of dopaminergic neurons in the substantia nigra pars compacta. One of the pathological hallmarks of PD is the presence of Lewy bodies, intracellular inclusions of aggregated alpha-synuclein. Although the cause of selective loss of dopamine neurons and the accumulation of alpha-synuclein in PD remain elusive, ongoing knowledge indicate that PD is the results of both environmental and genetic factors. The identification of alpha-synuclein aggregates within the degenerating
neurons has suggested that misfolded proteins represent a basic requirement for the neurodegenerative process and that dysfunctions of the protein catabolic systems play a crucial role in PD, together with mitochondrial dysfunctions, increased oxidative stress, glutamate excitotoxicity and neuroinflammation. Growing evidence in patient-derived samples and in animal and cellular models of PD have demonstrated the existence of dysfunctions in both degradative pathways (ubiquitin-proteasome system and autophagy-lysosome pathway) responsible for alpha-synuclein clearance. In this study, alterations of specific types and steps of the main autophagic pathways (macroautophagy and chaperone-mediated autophagy) involved in alpha-synuclein catabolism will be investigated and neuroprotective compounds or strategies will be tested in the attempt to correct these dysfunctions. These experiments will be performed in human neuroblastoma cell lines exposed to PD-related toxins or overexpressing wild-type or mutant alpha-synuclein, as observed in familial PD cases, and/or in human neurons obtained from induced Pluripotent Stem Cells (iPSCs) will be searched in patient-derived peripheral cells, possibly used as adjunctive tools for PD diagnosis and for monitoring the efficacy of therapies during the course of the disease.