CRCM

Propositions de sujets de stage de Master

2019 - 2020
Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Pancreatic Cancer
Name of the team/platform manager: J Iovanna

Supervisor name: Frederic André
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phone number: 

Number of PhD students in the team: 12 (1 in the group)
Number of M2 students in the team: 2 (0 in the group)
Number of M1 students in the team:

Title of the project: Cadherins as a signature of pancreatic cancer aggressivity.

5 keywords: cadherins, invasion, invadopodia, extracellular matrix, metalloprotease

Project summary (1 page max):

The low survival rates associated with Pancreatic ductal adenocarcinoma (PDAC) is due to a diagnosis on the later stage of the disease when local invasion and metastasis to other organs are already developed (1). Moreover, the tumor heterogeneity observed in PDAC seems to be responsible for inconstant response to treatment. Therefore, it is essential to characterize new molecular signatures to classify patients not only to predict clinical outcomes but also to propose individual treatment.

Our results based on whole gene and protein expression on 55 PDAC samples shows that expression levels of at least 5 cadherins impressively correlate with high evolution of the disease and consequently a poor survival (2). This suggests that these cadherins may represent potential targets for diagnosis, prognosis and new therapeutic treatment.

In this M2 project, we will confirm our observation by evaluating cadherins expression and localisation in the tumor tissues from a large cohort of PDAC. Expression levels will be correlated to the patient clinicopathological parameters and postoperative survival times.
To determine whether cadherins expression represents a signature of PDAC aggressiveness, we will conduct an invadomic analysis including 3D and 2D *in vitro* invasion assays and invadopodia assay. We will use cell models where the expression of the cadherins can be manipulated. Since cell invasion is a complex and multifactorial process that requires cell-cell adhesion, cell-extracellular matrix adhesion, extracellular matrix proteolysis and cell migration, we will therefore dissect the role of cadherins during these crucial events.

This project will open new avenues towards uncovering innovative options for diagnosis and targeted therapy of pancreatic cancer

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team: Leuko/Stromal interactions in normal and pathological hematopoiesis
Name of the team manager: M. Aurrand-Lions

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Number of PhD students in the team: 4
Number of M2 students in the team: 2
Number of M1 students in the team: 2

Title of the project: Adjuvant therapeutic targeting of leuko-stromal crosstalks in acute leukemia

5 keywords: Acute Myeloid Leukemia, B cell Acute Lymphoblastic Leukemia, Mouse models, xenografts

Project summary (1 page max):

Acute leukemia exists in different flavors depending on the hematopoietic lineage that is affected. In our team, we study two types of acute leukemia: B-cell lymphoblastic (B-ALL) and myeloid (AML). In both pathologies, we have identified signaling pathways that provide leukemic cells with survival signals which result in increased drug resistance (De Grandis & al, Canc Res, 2017; Aurrand-Lions & Mancini, Int J Mol Sci, 2018). Such pathways are controlled by the bone marrow microenvironment that can be reconstituted in vitro in order to test new adjuvant therapies targeting drug resistance pathways (Balzano & al, Cell Reports, in press). The candidate will work on this project and establish co-culture models in which new therapeutic agents will be tested. The project will be conducted in close collaboration with Ph.D students from the team that are working with mouse models (Stavropoulou & al, Canc Cell, 2016; Jamrog & al, PNAS, 2018).
References:
1-5


Proposed project for a Master 2 traineeship at the Cancer Research Center of Marseille

Name of the team/platform: MOLECULAR MECHANISMS OF TUMOR CELL MOTILITY
Name of the team/platform manager: Ali Badache

Supervisor name: Ali Badache; email:ali.badache@inserm.fr; phone number: 04 86 97 73 21

Number of PhD students in the team: 0; number of M2 students in the team: 1; number of M1 students in the team: 1

Title of the project: Characterization of protein complexes regulating microtubule capture, tumor cell division and motility

Keywords: migration, mitosis, cytoskeleton, macromolecular complexes, microscopy

Project summary:
Tumor cells escape controlled proliferation to form the primary tumor. Yet, the death of cancer patients is often the consequence of cancer cell migration from the primary tumor to distant sites, to form metastases. The work of our team aims to better understand this process, particularly in the context of breast cancer, with the aim of encouraging the emergence of novel therapeutic strategies targeting metastasis.

The actin cytoskeleton is known as one of the main players in cell migration. However, the contribution of microtubules, another major element of the cytoskeleton, remains much less known. Our past work has identified a signaling pathway that controls microtubule stabilization at the cell leading edge and has demonstrated its role in determining the direction of migration. Our current projects aim to identify and characterize, at the structural and functional levels, protein complexes that control the dynamic properties of microtubules and therefore cell migration, but also other fundamental processes such as mitosis. More recently, we have been interested in septins, poorly known constituents of the cytoskeleton, which appear as regulating elements of the oncogenic process and especially of cell migration; another of our objectives is to determine how septins ability to interact with actin filaments and/or microtubules contributes to their mode of action.

To achieve our objectives, we implement global approaches, such as interactomics, which allows us to systematically identify all the partners of a given protein and to define functional macromolecular complexes (or “molecular machines”); we use molecular and structural approaches to specify the interactions between proteins within complexes, which determine their mechanism of action; and finally, we employ cell biology approaches, including high-resolution microscopy and real-time microscopy and micropatterning, to visualize the impact of these complexes on biological processes, at the cellular and subcellular levels. Using this type of methodological approaches, the Master fellow will contribute to characterize the organization of a newly discovered protein complex that regulates microtubule dynamics and its role in division and migration of tumor cells.
By studying the mechanisms that coordinate the organization of the cytoskeleton, actin filaments, microtubules and septins, we hope to be able to highlight fundamental processes that allow the tumor cell to divide and migrate, and promote the identification of novel targets for future therapies.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Machine Learning for Precision Oncology and Drug Design

Name of the team/platform manager: Pedro Ballester

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Number of PhD students in the team: 3
Number of M2 students in the team: 0
Number of M1 students in the team: 0

Title of the project: Integrating machine learning with feature selection to predict treatment response from high-dimensional tumour profiles

5 keywords: machine learning, feature selection, personalised oncology, bioinformatics, omics

Project summary (1 page max):
A major challenge in biomarker discovery is the high-dimensionality of multi-omics data such as DNA methylation or miRNA profiles of tumours. Indeed, Machine Learning (ML) classifiers build with few data instances (e.g. 50-100) and a much higher number of features (e.g. ~20000) typically overfit these datasets. To enhance the performance of ML algorithms to cope with high-dimensional data, we have designed a strategy termed Optimal Model Complexity (OMC). One route to reduce dimensionality is to use more training data, but these are usually not available. An alternative route is to only consider the most informative features in the data, thus typically discarding the many thousands of less informative features (hence strongly reducing data dimensionality while retaining most the initial information content). However, the optimal subset of features depends on various factors (treatment, profile, cancer type and data set). OMC rigorously identifies this subset for each case via cross-validations. We have recently written a paper applying this Feature Selection (FS) technique to high-dimensional data: https://www.biorxiv.org/content/10.1101/277772v2

This internship will investigate the optimal integration of ML with FS for this class of problems. The initial application of OMC combines Random Forest (RF) with this univariate FS, which on average performs better than RF alone (i.e. embedded FS). Here we will combine the above ML
algorithms with more elaborated forms of FS such as Recursive Feature Elimination, multi-variate filters or other embedded FS, as evaluated in similar classification problems (Haury et al., 2011). Next, we will evaluate their performance across several types of tumours profiles. Synthetic data sets will be also analysed to understand how the number predictive features, the total number of features or the number of data instances influence the considered methods.

References:
Object: Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Predictive oncology
Name of the team/platform manager: Drs D BIRNBAUM / F BERTUCCI
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Number of PhD students in the team: 8 PhD
Number of M2 students in the team: 3 x Sc M2 - 2 x MD M2
Number of M1 students in the team: 0

Title of the project: Modeling and Targeting Advanced tumors

5 keywords: Solid tumors, drug resistance, drug targeting, biomarkers, translational research

Project summary

Our aim is to understand and fight aggressive cancers, to find new prognostic markers, new predictive markers of therapeutic resistance, new therapeutic targets, and to transfer the results to the clinic as an approach of Precision Oncology.

This is achieved in our team through three axes:

1- **A better characterization of tumors heterogeneity**
   This has helped us in the past to find new prognostic markers and novel therapeutic targets. Most importantly, this allows us to classify patients and to anticipate tumors' behavior. This has implications on patients' therapeutic management. We are pursuing with this approach, now using the most recent technologies and finest analysis tools (NGS, RNA-seq and single cell analyses).

2- **New biomarkers, new predictors and next generation modelling of aggressive cancers to anticipate metastatic evolution (E Mamessier / C Acquaviva)**
   We are studying circulating tumor cells as quasi-noninvasive biomarkers that can be used to monitor the progression of a tumor in real-time. The characterization of those cells allows us to understand key biological mechanisms of metastatic development and progression. In parallel, we are developing organoid-based pre-clinical models, more or less complex, to study tumor behavior progression in response to treatment in a time frame compatible with aggressive disease development. This innovative model will be used to study drug resistance mechanisms.
3- **New treatment strategies and new targets for a better treatment (P Rocchi / M Lopez)**

The first axis provides target candidates and the second axis biomarkers and models to study tumor progression. The aim of the third axis is to develop novel specific tools targeting specific subsets of aggressive tumors. We use antisense oligonucleotides and novel or repositioned targeted therapeutics, with the prospect of transferring them to the clinic. Our facilitated access to clinical samples, human-derived models and most advanced technologies help in the validation of these novel specific tools.

These three approaches are developed in our laboratory through tight and overlapping collaborations of dedicated experts for each topic. The Master 2 student will work on one of the last 2 axes, depending of his/her wishes or available subjects at the time. One major asset of our laboratory is to provide a broad overview and strong expertise on most recent technics and concepts used in pre-clinic studies.

**References (since 2018 and restrained to solid tumors only):**


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Cell polarity, Signaling and Cancer
Name of the team/platform manager: Pr Jean-Paul Borg
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Number of PhD students in the team: 4
Number of M2 students in the team: 1
Number of M1 students in the team: 1

Title of the project: Study of novel components of the tyrosine kinase receptor PTK7 signaling

5 keywords: cell polarity, tumorigenesis, tyrosine kinase, PTK7, proteomics

Project summary (1 page max):

Tumorigenesis is frequently associated with alterations of tissue architecture, and increased cell migration and invasiveness of tumoral cells, leading to metastasis. Our lab focuses on the study of cell polarity proteins in physiology and oncology. We study the non-canonical Wnt signaling pathway involved in planar cell polarity (PCP) which is crucial during embryogenesis and altered in many cancers (1). We recently characterized two poorly described membrane receptors (PTK7, VANGL2) for their implication in Wnt/PCP pathway and tumor invasion. Our research focus on the mode of action of these receptors and their associated signaling molecules using state-of-the-art proteomics and cell biology methods, on their implication during the tumorigenic process using in vivo approaches, and on the design of therapeutic strategies (2).

We have contributed to the identification of novel partners of VANGL2 and its associated adaptor PRICKLE1 which make unexpected links between these WNT/PCP components and the p62/SQSTM1 (3) and mTORC2 (4) signaling molecules. Importantly, we linked alterations of these novel networks to poor prognosis in breast cancers, opening potential avenues in cancer treatment.

PTK7 is overexpressed in various human solid and hematological cancers which correlates to poor prognosis and to increased resistance to chemotherapeutic agents (5-8). We recently defined PTK7 as a poor prognosis marker as well as a pro-migratory and pro-metastatic receptor in colorectal cancer (7). A recent report demonstrated that expression of PTK7 is high on tumor-initiating cells of lung, ovarian and triple-negative breast cancer patient-derived xenografts and immune cells (PDXs), and targetable by novel drugs (9). Using proximity biotinylation coupled to mass spectrometry, we have recently defined totally novel protein networks associated to PTK7 in colon cancer cells. The selected Master student will further investigate the novel PTK7 interactors using functional assays and explore the PTK7 interactome
in immune cells. This project will benefit from strong collaborations with leading national and international teams in basic and translational research.

References:

Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Antibody Therapeutics and Immunotargeting
Name of the team/platform manager: Patrick Chames

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Number of PhD students in the team: 2
Number of M2 students in the team: 1
Number of M1 students in the team: 0

Title of the project: Generation of bispecific immune checkpoint inhibitors using nanobodies for cancer immunotherapy

5 keywords: immunotherapy, antibodies, bispecific, cancer, NK cells,

Project summary (1 page max):
The recent successes of therapeutic antibodies called immune checkpoint inhibitors (ICIs) blocking inhibitory receptors found on tumor-infiltrated immune cells have revolutionized the therapy of several cancers, to the point that immunotherapy is now considered the fourth pillar of cancer therapy together with surgery, radio and chemotherapy, and is actively being developed at IPC/CRCM. Still, only a minority of treated patients benefit from those treatments, and there is an urgent need to expand the arsenal of ICIs. Beside the classically targeted T cells, natural killer (NK) cells are reportedly important actors of the anti-tumor immune response, through both killing directly tumor cells and secreting several cytokines and chemokines that can recruit and activate cells of the innate and adaptive immune systems. Interestingly, NK cells also express inhibitory receptors amenable to blockade, and anti-KIR and NKG2A mAbs are currently in clinical trials. Less studied inhibitory receptors expressed on NK cells are TIGIT, and CD96. Our objective is to explore the possibility of blocking these receptors, using multispecific nanobody-based molecules enabling mode of actions not afforded by conventional antibodies.

We have already demonstrated the possibility to outperform the ADCC mode of action of conventional mAbs such as trastuzumab by targeting the activating CD16 receptor notably expressed by NK and macrophages, using small bispecific antibody fragments generated using single domain antibodies or nanobodies, i.e. the variable domain of antibodies naturally devoid of light chain found of Camelids.

In this emerging project, our aim is to exploit this know-how to generate NK cell-targeting bispecific ICIs with innovative mode of actions. Two inhibitory receptors, CD96 and TIGIT, expressed by NK but also T cells, are interacting with the same ligand CD155. They inhibit NK activity, directly through recruitment of phosphatases at the immunological synapse, but also by directly competing for binding to CD155 with DNAM-I, one of the main NK activating receptors. Our objective is to generate small bispecific/biparatopic antibodies able to generate an
avidity effect by simultaneously blocking these two receptors, either by direct competition with the ligand, or by trapping these two receptors in an interaction-incompetent state. A number of blocking nanobodies have been isolated and are being currently characterized. The specific aim of the M2 student will be, using these nanobodies as building blocks, to design and produce multispecific/multivalent constructs, produce and purify them using antibody engineering approaches (cloning, production in eukaryotic cells, affinity purification, SDP-Page and western blots). Next, the student will have to characterize these new molecules in terms of affinity, specificity using flow cytometry. Finally immunological cellular assays will be used to characterize the functionality of these new therapeutics (cell culture, cytokine secretion, follow up of activation marker by flow cytometry, cytotoxicity assays).

References:
Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Epithelial Stem Cell and Cancer
Name of the team/platform manager: Pr E Charafe-Jauffret/Dr C Ginestier
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Number of PhD students in the team: 3 PhD
Number of M2 students in the team: 2 M2
Number of M1 students in the team: 1 M1

Title of the project: A chemical biology strategy to unravel molecular targets of repurposed drug as a potential cancer stem cells targeted therapy

5 keywords: cancer stem cell, drug repositioning, click chemistry, breast cancer, targeting

Project summary (1 page max):

Epithelial cancers are heterogeneous due a hierarchical organization of tumor tissues where several subpopulations of self-renewing cancer stem cells (CSCs) sustain the long-term oligoclonal maintenance of the neoplasm, drive disease progression and fuel therapeutic resistance. Thus, developing CSC-targeting therapies is of major interest to improve cancer care. In order to identify new anti-CSC therapies, we carried out a chemical library screen to identify FDA-approved compound able to target efficiently the CSC population. We identified Nifuroxazide (NIF), a 5-nitrofuran antibiotic widely used in human for over 40 years, with a potent anti-CSC effect in breast, colon, pancreatic, and gastric cancer models. Recently it has been confirmed as having an anti-CSC activity in melanoma, acting as a toxic suicide substrate for ALDH1A1 and 1A3 enzymes. These enzymes are preferentially expressed in CSC subpopulations. However the molecular mechanism sustaining the CSCs killing effect of this compound remains unknown as well as the opportunity of NIF positioning as adjuvants to prevent breast cancer relapse.

The aim of this project is to use NIF clickable analogs (synthetized in Institut Curie by R Rodriguez’s team). These analogs are designed to preserve NIF functional integrity but amenable to bio-orthogonal chemical ligation in order to decipher nifuroxazide killing molecular mechanism of action in cancer stem cells. Then, we will set up pre-clinical trials to challenge NIF as a valid therapeutic approach to eradicate the CSC burden and overcome tumor relapse in combination with conventional treatments. The final aim, in the scope of personalized medicine, is to determine if NIF could be re-positioned in clinic as a valuable adjuvant therapy to prevent cancer relapse.
References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): TrGET preclinical assay plateform
Name of the team/platform manager: Yves Collette
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Number of PhD students in the team: 0
Number of M2 students in the team: 1
Number of M1 students in the team: 0

Title of the project: Experimental modeling of Acute Myeloid Leukemia and chemo-genomic approach characterizing the molecular and functional basis of resistance to standard treatment for the identification of innovative therapeutic approaches.

5 keywords: Acute Myeloid Leukemia (AML), preclinical study, chemo-genomic approach

Project summary (1 page max):

Acute myeloid leukemia (AML) affects nearly 3,000 new patients each year in France and ranks fifth among common malignant hemopathies. With current treatments (mainly based on chemotherapy), the 5-year overall survival rate for these patients is only 27%. More effective treatments are needed. Recent genomic and molecular characterization has revealed the generalized heterogeneity of (AML without explaining the variability of results after conventional treatment. A systems biology approach is needed to establish groups of patients that correlate with a particular therapeutic response and identify deregulated signaling pathways.

We have implemented an integrated biology approach based on the combination of preclinical data (search for genomic abnormalities, ex vivo susceptibility / resistance profiling to a panel of 80 clinically approved and / or in development drugs - DSRP) and clinical trials to characterize AML patient specimens collected in 2 open-label and active clinical trials (HEMATO-BIO-IPC 2013-015 / NCT02320656 and CEGAL-IPC-2014-012 / NCT02619071). Our preliminary statistical and bioinformatic analyzes obtained from more than 150 AMLs to date have allowed us to identify drug response patterns associated with certain molecular alterations, or the "clustering" of drugs with similar profiles suggesting mechanisms of action comparable and / or susceptible to be used in combination. Some of the patients relapsed after their treatment and included in the CEGAL study were previously included at diagnosis in the HEMATO-BIO study, and there are therefore in our annotated collection "pairs" of samples from the same patient (Diagnosis then relapse) which we can compare to evaluate between the evolution of genomic abnormalities and ex vivo responses to the drug panel in parallel with the evolution of his disease. These data thus suggest a functional link between the gain (or loss) of sensitivity to certain drugs and the genomic abnormalities
that occurred (or were selected as a result of treatment) between the diagnosis and the relapse that we propose to evaluate during this project as a causal link and/or as potential biomarker of response to some of these drugs. This cross-cutting project is based on solid foundations and established technologies available in the laboratory. It opens diverse and important innovative perspectives in the field of the translational research of AML, aiming to identify new therapeutic tracks by repositioning of existing drugs, and/or identification of associated targets and/or biomarkers, in the particularly concerning situation of relapse or resistance to standard treatments currently available. Exploration of the associated molecular and mechanistic bases should also contribute to a better understanding of this disease and its evolution.

References:


Proposed project for a Master 2 traineeship at the Center for Cancer Research of Marseille

Name of the team:

Telomere and Chromatin

Name of the team manager:

Vincent Géli

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Number of PhD students in the team: 3
Number of M2 students in the team: 2
Number of M1 students in the team: 1

Title of the project:

Role of the Replication Protein A (RPA) in telomere maintenance: from molecular mechanism to telomere diseases.

5 keywords:

Telomeres, Replication stress, G-quadruplex, Telomere syndrome, Pulmonary Fibrosis

Project Summary:

Telomeres are key features of linear chromosomes that preserve genome stability. In mammals, they are composed of short tandemly TTAGGG repeated DNA sequences ending with a 3’ single-stranded DNA overhang on the G-rich strand. The telomeric sequences are protected by the shelterin complex. In the absence of special telomere maintenance mechanisms, linear chromosomes shorten with every round of DNA replication, leading to replicative senescence or apoptosis. Thus, telomere replication is a crucial step in maintenance of chromosomes ends, genome stability and tumorigenesis.

We recently reported the role of the Replication Protein A (RPA) complex, a highly conserved heterotrimeric ssDNA binding protein, in telomere replication in yeast. Interestingly, RPA prevents telomere attrition by unwinding DNA secondary structures, such as G-quadruplex, during telomere replication. Additionally, we have isolated mutations in genes encoding for the subunits of RPA complex in patients developing idiopathic pulmonary fibrosis (IPF), a clinical feature of telomeric disease. These
clinical cases strengthen our hypothesis that RPA is required at telomeres to avoid fork replication breakage and telomeric alterations during replication.

The object of this project is to link the Replication RPA to telomere functions and to genome instability, and also to determine if alteration of this complex is responsible for human diseases. To achieve this goal, we will introduce mutations found in RPA patients into mammalian cell lines and study their impact on genome stability and on telomere maintenance. We expect to shed light on new functions of RPA on telomere maintenance. We predict that mutations that alter RPA functions may participate to genome stability and tumorigenesis.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team: Signaling, Hematopoiesis and Mechanism of Oncogenesis
Name of the team manager: Paulo De Sepulveda / Patrice Dubreuil

Supervisor name: Paulo De Sepulveda
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Number of PhD students in the team: 2
Number of M2 students in the team: 3
Number of M1 students in the team: 0

Title of the project: Novel links between FLT3-ITD oncoprotein and metabolic pathways in AML

5 keywords: leukemia, kinase, metabolism, therapeutic target, RNAi

Project summary (1 page max):

FLT3 is a major oncoprotein in acute myeloid leukemia. FLT3 is one of the most frequent mutated genes in AML, present in at least 25% of patients. The mutation status alone is a prognosis factor of disease relapse and poor survival. Activated FLT3 elicits pleiotropic cell responses. In addition to the activation of classical signaling pathways (MAPK, PI3K, STAT), oncogenic FLT3 activates proteins and pathways which are poorly delineated to date.

Oncogenic FLT3 has been recently involved in the regulation of cell metabolism. For instance, FLT3 regulates glycolysis through increased absorption of glucose and through activation of Hexokinase 2, the first enzyme of glycolysis. Our laboratory is studying oncogenic FLT3, with a focus on discovering novel therapeutic targets in FLT3 mutated AML cells. Since cell metabolism is a hallmark of cancer and, metabolic enzymes are mutated in blood neoplasms, we are studying the connection between oncogenic FLT3 and enzymes involved in metabolic pathways.

Previous RNAi screens performed in our lab have identified critical genes that are essential in an AML cell line with endogenous FLT3 mutation but not in other AML cells. Using exome sequencing, we have also found metabolic enzymes with upregulated expression in FLT3 mutated AML cells.

The Masters’ research project will consist in targeting several of these genes both using RNAi and chemical inhibitors when available, in order to: (1) confirm the specificity of the FLT3 dependence on other cell lines and primary patient samples, (2) to delineate the molecular links between FLT3 receptor and the enzyme, and (3) investigate the importance of the metabolic pathway corresponding to the selected enzymes.
References:
Lortholary O, M O Chandesris, C Bulai Livideanu,.., A Moussy, P Dubreuil, O Hermine


Zorzan E, Hanssens K, Giantin M, Dacasto M & P Dubreuil Mutational hostpots of TET2, IDH1, IDH2, SRSF2, SF3B1, KRAS, and NRAS from human systemic mastocytosis are not conserved in canine mast cell tumors. Plos One, 2015 10(11):e0142450


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Epigenetic control of normal and pathological hematopoiesis

Name of the team/platform manager: Estelle Duprez

Supervisor name: Estelle Duprez

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Number of students in the team: 1 PhD, 1 M2, 0 M1

Title of the project: Histone genes in normal hematopoiesis and leukemia.

5 keywords: Epigenetics, Histone, leukemia, transcription, CRISPR/Cas9

Project summary (1 page max):

Using epigenetic profiling, our group has discovered a new epigenetic alteration referred as H3K27me3 HIST1\text{high} that affects AML with normal karyotype (CN-AML) and impacts on prognosis. This epigenetic alteration is characterized by a striking enrichment of the repressive histone mark on chromosome arm 6p that spans 70 kb of the HIST1 locus. It is associated with the presence of an NPM1-mutated allele (NPM1\text{mut}) but not with known mutations in chromatin modifiers and predicts a good prognosis (Tiberi, leukemia, 2015 and Patent WO2015169906-A1). Further analyses have shown that H3K27me3 HIST1\text{high} can distinguish patients with a less aggressive and more mature disease suggesting that this epigenetic mark globally influences molecular and cellular processes. We have also investigated at the molecular level the consequences of this H3K27me3 islet and demonstrated that patients bearing the H3K27me3 HIST1\text{high} mark express low levels of histone. (Garciaz, submitted and second patent deposition). The proposed project will be a direct follow up of these studies and will aim to understand the effect of H3K27me3 HIST1\text{high} on chromatin and biological properties of normal hematopoietic and leukemic cells. The student will generate/study different KO cellular models in collaboration with team members using CRISPR/cas9 technologies aiming to model H3K27me3 HIST1\text{high}. These modified cell lines will be used to test the effect of part of the HIST1 locus deletion on cellular proliferation, leukemia development and treatment response. In particular, chemotherapeutic and epigenetic drugs, alone and/or in combination will be tested on these modified cell lines. These biological effects will be analysed in parallel with chromatin structure changes by looking for epigenetic histone marks, CTCF localisation and long-range interactions, in order to assess the consequences of topology association domain (TAD) disorganization under HIST1 deletion.

Understanding on how the HIST1 locus is central to chromatin regulation and leukemic phenotype will help to define risk factors for AML evolution and new therapeutic target that will help for therapeutic decision. As consequence of H3K27me3 HIST1\text{high}, the M2 work will pinpoint the importance of histone gene in acute myeloid leukemia.
References:

Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Control of structure-specific endonucleases and genome stability
Name of the team/platform manager:

Supervisor name: Pierre-Henri GAILLARD
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06 30 48 40 45

Number of PhD students in the team: 1
Number of M2 students in the team:
Number of M1 students in the team:

Title of the project: Control mechanisms of the Mus81-Eme1 endonuclease to prevent chromosomal translocations

5 keywords:
DNA repair, Bloom syndrome, cell-cycle control, DNA damage checkpoints

Project summary (1 page max):

Structure-specific endonucleases are specialized surgical tools that the cell uses to process secondary DNA structures. Control of these enzymes is pivotal to ensure that they do not become a major source of chromosomal instability that could fuel tumorigenesis (Dehé and Gaillard, 2017). The Mus81-Eme1 complex is a DNA endonuclease specialized in the processing of various DNA secondary structures including complex structures established during homologous recombination between homolog chromosomes and sister chromatids. In both budding and fission yeast, Mus81-Eme1 is essential for cell viability in absence of the Sgs1 and Rqh1 RecQ helicases, respectively. Importantly, these are orthologs of the human BLM helicase that is defective in Bloom syndrome patients, a hereditable syndrome associated with remarkable chromosomal instability and cancer predisposition.

Our research has led to the discovery of a novel DNA damage-mediated control of Mus81-Eme1 in fission yeast that is necessary to maintain chromosome stability in absence of Rqh1^{BLM}. This regulation relies on a cell-cycle driven phosphorylation of Eme1 by Cdc2^{CDK1} that primes Eme1 for phosphorylation by Chk1 following DNA damage, ultimately resulting in catalytic stimulation of Mus81-Eme1 in G2/M in response to DNA damage. Importantly, our findings have allowed us to engineer unprecedented separation of function eme1 mutants where we have specifically impeded a
function of Mus81-Eme1 that is essential in absence of Rqh1\textsuperscript{BLM} but totally dispensable for the cellular response to genotoxic drugs such as hydroxyurea, camptothecin or MMS to which mus81\textDelta and eme1\textDelta null mutants are usually exquisitely sensitive (Dehé et al., 2013).

In an effort to further dissect the control mechanism of Mus81-Eme1 we have identified two SUMO interacting motifs (SIMs) at the N-terminus of Eme1. Both SIMs confer SUMO binding properties to Eme1. Phenotypic analyses of SIM mutants indicate that SUMO-binding is critical for cell fitness in absence of the Rqh1\textsuperscript{BLM} helicase. Strikingly, while the eme1\textsuperscript{sim} mutants are unable to undergo DNA damage-induced phosphorylation, they can still undergo the first Cdc2\textsuperscript{CDK1}-dependent wave of phosphorylation indicating that they are specifically impacted in the DNA damage-induced secondary wave of phosphorylation. These findings unravel a remarkably complex regulatory network of the Mus81-Eme1 endonuclease that relies on a functional link between association of Eme1 with one or several SUMOylated partners and DNA damage-induced phosphorylation. They also provide us with a different set of separation of function mutants, which will considerably help us dissect the molecular mechanisms underlining the control of Mus81-Eme1.

Most importantly, we find that impeding the SUMO-binding functions of Eme1 in absence of a functional Rqh1\textsuperscript{BLM} helicase leads to large chromosomal translocations that are initiated at sites of retrotransposons. Such translocations are reminiscent of those found in cancerous cells. We plan to further investigate the underlying molecular mechanisms leading to those translocations in fission yeast and to exploit the knowledge acquired with this model organism to expand our analyses to human cells and assess their relevance in terms of cancer development.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Control of structure-specific endonucleases and genome stability
Name of the team/platform manager:

Supervisor name: Pierre-Henri GAILLARD
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Number of PhD students in the team: 1
Number of M2 students in the team:
Number of M1 students in the team:

Title of the project: Functional studies on the SLX4 tumour suppressor: from patient derived mutations to synthetic lethal drug screening for new therapeutical strategies

5 keywords:
Cell cycle control and DNA repair, Breast cancer, Blood related disorders, Fanconi anemia

Project summary (1 page max):

The human SLX4 protein is a tumour suppressor that is mandatory for genome stability, notably through its interactions with numerous DNA repair factors and other partners involved in genome maintenance. Bi-allelic mutations in SLX4 are causative of Fanconi Anemia, a cancer-predisposing syndrome. There is accumulating evidence that heterozygous mutations in SLX4 are found in familial breast cancer while numerous SLX4 mutations are present in various cancer cell lines originating from different organs. In agreement, mutations in the SLX4 gene have been identified in a significant cohort of patients treated at IPC. Our laboratory has an ongoing collaborative project with the laboratory of Molecular Oncology headed by Dr Daniel Birnbaum on the functional analysis of cancer-associated SLX4 variants.

Our team identified the human SLX4 protein a decade ago and is since one of the leaders on the functional characterization of this fascinating scaffold protein (Crossan et al., 2011; Fekairi et al., 2009; Guervilly et al., 2015). SLX4 is involved in the control of at least three different structure-specific endonucleases. These can be viewed as specialized surgical tools that the cell uses to process secondary DNA structures. Control of these enzymes is pivotal to ensure that they do not
become a major source of chromosomal instability that could fuel tumorigenesis (Dehé and Gaillard, 2017). Importantly, SLX4 also interacts with other key factors involved in DNA repair and cell-cycle control (Guervilly and gaillard, 2018) and is emerging as an important factor that coordinates DNA repair with DNA replication and chromosome segregation.

Our laboratory currently offers several projects that are aimed at understanding how SLX4 fulfils its anti-tumoral functions as well as how it may be targeted in novel therapeutic strategies by screening for synthetic lethal drugs that specifically kill cells producing SLX4 loss of function mutants.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Spatio Temporal Regulation of Cell Signaling – Scaffolds & Phosphoinositides.
Name of the team/platform manager: Pascale ZIMMERMANN

Supervisor name: Rania GHOSSOUB
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Number of PhD students in the team: 0
Number of M2 students in the team: 1
Number of M1 students in the team: 1

Title of the project: Syndecan / Tetraspanin networks in molecular sorting and cell signaling.

5 keywords: cell biology, molecular biology, scaffolds, membrane trafficking, microscopy

Project summary (1 page max):

Cell signaling is regulated by the confinement of signaling receptors in membrane compartments. A better characterization of the molecular mechanisms that regulate these processes is essential to our understanding of life and would bring new hopes in therapeutics including cancer. Syndecans (SDCs) and Tetraspanins (TSPNs) are transmembrane proteins that dictate the destination and location of many signaling receptors. SDCs directly interact with growth factors and adhesion molecules through their heparan sulfate chains. TSPNs form a family of 33 proteins that are known to coordinate membrane protein networks. The TSPNs regulate the traffic and the signaling of numerous adhesion molecules and receptors with growth factor. We highlighted a TSPNs-SDCs collaboration. The project aims to better understand how SDCs and TSPNs communicate to regulate vesicular trafficking and thus associated receptor activity. The candidate will evaluate the impact of SDCs / TSPNs networks on cell signaling and cancer biology and will become familiar with molecular cloning, cell culture, protein loss and gain of function, and microscopy experiments.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Epithelial Stem Cells and Cancer
Name of the team/platform manager: Emmanuelle Charafe-Jauffret/Christophe Ginestier

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Number of students in the team: 3 PhD, 2 M2, 1 M1

Title of the project: Modelisation and targeting of cell plasticity during breast cancer chemoresistance

5 keywords: Breast cancer, Therapeutic resistance, Cancer stem cells, Cell plasticity, Lineage tracing

Project summary (1 page max):

Breast cancers are known to present a major intratumoral heterogeneity that contributes to therapy failure and disease progression. It is now well accepted that neoplasms change over time through an evolutionary process at the cell level, driven by genetic and epigenetic alterations, and especially under sustained treatment (Maley et al, 2017). This evolution explains the processes of tumor progression and therapeutic resistance. While tumor cell heterogeneity composes a complex ecosystem, cancer stem cells (CSCs) seem to orchestrate the evolutionary selection in cancer (Greaves, 2015; Kreso & Dick, 2014). Breast CSCs are relatively resistant to conventional therapies (radiotherapy and chemotherapy) and, CSCs are thought to be the seed for the distant metastasis responsible for poor clinical outcome (Oskarsson, Cell Stem Cell, 2014). Interestingly, it has been proposed that the tumor bulk can fuel the CSC pool under treatment pressure. Hence, this treatment-induced cell plasticity drives the emergence of reprogrammed CSC leading to tumor relapse. Understanding the molecular mechanisms leading to this adaptive resistance represent a prerequisite to improve breast cancer care.

In the lab, we have developed a lineage tracing system to monitor in vitro the cell-state dynamics during therapy. We proposed to explore the cell plasticity for a series of breast cancer models in response to a panel of therapies (conventional chemotherapies and targeted therapies). Using scRNAseq we want to identify mechanisms responsible to this treatment-induced cell plasticity and proposed new therapies to block this cell reprogramming and prevent tumor relapse.
References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team:

Homologous Recombination, NHEJ and Maintenance of Genomic Integrity

Name of the team manager:

Mauro MODESTI

Supervisor name: Stéphanie GON
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Number of PhD students in the team: 1
Number of M2 students in the team: 1
Number of M1 students in the team: 0

Title of the project:

Role of the RAD51 Paralogs in Cell Cycle Regulation in Response to DNA Damage

5 keywords:

Homologous Recombination, RAD51, RAD51 paralog, RAD51B, RAD51C, RAD51D, XRCC2, XRCC3

Project summary

Recently, mutations in the classical RAD51 paralog genes \([RAD51B, RAD51C, RAD51D, XRCC2] and XRCC3\) have been linked to predisposition to breast, ovarian or other cancers. Moreover, hypomorphic mutations in \(RAD51C\) and \(XRCC2\) confer Fanconi’s anemia and are now named FANCO and FANCU, respectively.

Using CRISPR-Cas9 genome editing, our team has generated collections of isogenic human cell lines in which the five classical RAD51 paralogs have been individually disrupted. These lines are important tools to study the role of the RAD51 paralogs in genome maintenance mechanisms.

In this project, we will focus on determining the role of the RAD51 paralogs in cell cycle regulation in response to DNA damage. One specific aspect will be to explore their role in checkpoint functions.
Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Spatio Temporal Regulation of Cell Signaling – Scaffolds & Phosphoinositides.
Name of the team/platform manager: Pascale ZIMMERMANN
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Number of PhD students in the team: 0
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Number of M1 students in the team: 1

Title of the project: Exploring the importance of syntenin from blasts for acute myeloid leukemia progression

5 keywords: leukemia, exosomes, biomarker, cell biology, in-vivo models.

Project summary (1 page max):

1. Concept. Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells. Over 20,000 new cases are diagnosed worldwide per year, with a 5-year survival rate below 30%. This highlights a need for research into the mechanisms supporting AML and causes of therapy failure. Leukemias are characterized by the clonal expansion of hematopoietic cells at distinctive stages of differentiation and origin from leukemic stem cells (LSCs) located within the bone marrow (BM). Within a leukemic niche, leukemic cells both hijack/invade the hematopoietic stem cell niches and instruct/educate BMSCs to support leukemia. While direct cellcell contact, and conventional chemokine and cytokine mediated interactions are all known contributors to leukemia stroma crosstalk, these fail to fully explain how leukemic invasion results in niche remodeling. Currently, the notion that nanoparticles called exosomes play an important role in ‘cancer crosstalk’ is widely accepted (Kumar et al., Leukemia 2018). Different types of exosomes, which contain specific material that is commonly designated as cargo, can be isolated from body fluids. Cargo transfer to cells regulates gene expression and alters the fate of target cells. Although several studies have demonstrated the role of exosomes in the progression of AML, as well as AML resistance to treatment, the associated molecular mechanisms remain unknown.

2. Objectives. The biogenesis of exosomes remains poorly understood. The current hypothesis is that several separate sorting mechanisms drive the formation of distinctive exosome populations containing different cargo molecules. Our laboratory has played a pioneering role in this respect, identifying a molecular machine, supported by the PDZ protein syntenin, which regulates the biogenesis of a specific subclass of exosomes (Fares et al., Cell Adh Migr 2017 ; Imjeti et al., PNAS 2017). Syntenin is highly expressed during early development and its protein levels are abnormally elevated in several types of solid cancer (Melanoma, Breast cancer, Glioma and others). Some studies even propose that the levels of syntenin protein in the tumor might constitute a marker of disease aggressiveness. Interestingly, syntenin is also known to be involved in signaling pathways that regulate the hematopoietic compartment. We thus launched a study of syntenin in the progression of leukemia, a disease in which the role of syntenin was never investigated and remains to be established.
3. Methodology

By using both syngeneic and preclinical human models of AML, with enhanced or depleted for syntenin expression, we will thus investigate, *in vitro* and *in vivo*, how syntenin levels in cells and exosomes evolve with leukemia aggressiveness.

Results obtained will be correlated/validated with a study exploring the prognostic significance of syntenin expression in patient samples. We then propose to ‘profile’ the syntenin levels in leukemic cells and circulating exosomes in patient samples. In this respect, we have access via the Paoli Calmettes institute to AML samples from 70 patients.

4. Expected Results. We aim to clarify the roles of exosomes and of syntenin in leukemia progression, obtain first information on their potential usefulness as biomarkers, possibly even identifying these as interesting novel molecular targets in therapeutic approaches. We expect high levels of tumor syntenin to be associated with the aggressiveness of the disease and a poor prognosis.

5. Impact. This project is important to clarify the value of syntenin as a ‘systemic’ target. We aim to better coin the potential relevance of antisyntenin therapy (i.e. small chemical compounds) for the treatment of acute myeloid leukemia. Syntenin might constitute a new biomarker of the aggressiveness of AML.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Genome dynamics and Recombination
Name of the team/platform manager: Bertrand Llorente

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Number of PhD students in the team: 3
Number of M2 students in the team: 0
Number of M1 students in the team: 0
Title of the project: Transform the mitotic Rad51 recombinase into a meiotic recombinase

5 keywords: recombination, mutagenesis, base mismatches, paralogous genes

Project summary (1 page max):

Homologous recombination is a ubiquitous DNA repair mechanism that consists of repairing DNA breaks by copying the genetic information of homologous loci. Recombinases are proteins of the RecA family, which include the Eukaryotic Rad51 protein, that are essential to mediate homologous recombination and more specifically to allow homology search and pairing between homologous sequences. Dysfunction of the homologous recombination machinery is frequently observed in cancer cells and responsible for the resulting genetic instability. Interestingly, Rad51 is responsible for mitotic recombination only, and the Rad51 paralog Dmc1 mediates meiotic recombination exclusively. A major difference between the two being that Rad51 is less tolerant to DNA mismatches than Dmc1 (Lee et al., 2017). This makes sense since mitotic recombination mainly involves sister chromatids of identical sequence, while meiotic recombination involves homologous chromosomes of different parental origins (mom and dad) that frequently show sequence polymorphisms. Interestingly, by manipulating yeast cells, it is possible to force meiotic recombination in the absence of Dmc1 and in the presence of Rad51, but only in a homozygous context. When such an experiment is carried out in a heterozygous context, spore lethality becomes extremely high, meaning that Rad51 is not able to support recombination in the presence of DNA mismatches unlike Dmc1 (Callender et al., 2016). Here, the plan is to mutagenize yeast Rad51 to find mutants that would allow recombination in the presence of DNA mismatches. This structure-function study will shed light on the functional specialization between Rad51 and Dmc1, and ultimately allow a better understanding of the regulation between homologous and homeologous recombination, which is highly relevant in the context of genomes containing highly repeated homeologous sequences such as the human genome.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

**Name of the team/platform (please specify):** Pancreatic Cancer (Eric Mas’ research group: Aberrant glycosylation processes in pancreatic cancer)

**Name of the team/platform manager:** Juan Iovanna

**Supervisor name:** MAS Eric
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**Number of PhD students in the team:** 10 for 13 Researchers ; (1 in the Eric Mas’ research group)
**Number of M2 students in the team:** 2 ; (0 in the Eric Mas’ research group)
**Number of M1 students in the team:** 0

**Title of the project:**
Deciphering the role of polypeptide N-acetylgalactosaminyltransferases 4 and 12 (GALNT4 and GALNT12) in pancreatic tumor progression.

**5 keywords:**
Pancreatic adenocarcinoma (PDAC) ; Glycobiology ; polypeptide N-acetylgalactosaminyltransferase 4 and 12 (GALNT4, GALNT12) ; tumor progression; metastasis.

**Project summary (1 page max):**
Pancreatic adenocarcinoma (PDAC) is a devastating disease progressing asymptptomatically until death within months after diagnosis. The predisposition to metastasize is very important in the development of PDAC and implicates complex molecular mechanisms. In particular, aberrant glycosylation of glycosphingolipids and glycoproteins expressed in tumor cells has been implicated as one of essential mechanisms in malignant transformation, cell adhesion and metastatic dissemination. Although it was shown that modified expression of glycosyltransferases and glycosidases play a key role in the formation of aberrant glycoconjugates, little information is available on the regulation of mechanisms that produce altered glycan structures during pancreatic carcinogenesis.

By means of the transcriptomic data obtained from Patient-Derived Xenografts (PDX) biobank, we have determined the expression profiles of genes encoding for glycosyltransferases. Among them, we have observed a deregulated expression of N-acetylgalactosaminyltransferases (GALNTs), a subfamily of glycoenzymes which catalyses the transfer of N-acetylglactosamine (GalNAc) to Serine or Threonine residues in the initial step of mucin-type O-glycosylation. Hence, these glycosyltransferases could
produce abnormal O-glycans and thereby play a key role in PDAC progression. In this context, we have identified a very poor pronostic PDAC subtype that is associated with GALNT4 and GALNT12 downregulation.

The aim of our study will be to evaluate the impact of GALNT4 and GALNT12 downregulation on pancreatic tumor progression. To determine if the GALNT4 and GALNACT12 mRNA and enzymes may serve as novel pronostic markers, the expression pattern of GALNT4 and GALNT12 will be determined by immunohistochemistry, RT-qPCR, western-blot, confocal microscopy and flow cytometry on PDX-Biobank (human tumor tissues, TMA and primary cell lines established from these tumors). The role of GALNT4 and GALNT12 will be characterized through their silencing and/or their overexpression using different technologies on appropriate primary cell lines from PDX-Biobank. The impact of these gene inactivation/overexpression on cell surface expression of glycoconjugates will be analyzed by western-blot, flow cytometry and immunofluorescence using specific antibodies and lectins. The impact on migration and invasion properties will be also determined in vitro using boyden chamber, wound healing migration assays, adhesion assays on endothelial cell lines (HUVEC ...), invasion assays and video microscopy. It will be also possible to validate these in vitro results in induced PDAC mouse model following orthotopic injection in nude NMRI mice of modified PDX-biobank cell lines inactivated and/or activated for GALNT4 and GALNT12. The photon Imager will be used as tools for non-invasive exploration of pancreatic tumor to monitor PDAC progression (primary tumor volume and metastatic dissemination).

In summary, the deregulated expression of GALNT4 and GALNT12 could contribute to the synthesis of cancer-associated aberrant O-glycans to promote PDAC progression. These 2 glycoenzymes could be potential targets to impair PDAC development.

References:
Proposed project for a Master 2 traineeship at the Cancer Research Center of Marseille

Name of the team/platform: **IMMUNITY & CANCER**
Name of the team/platform manager: **Jacques Nunès & Daniel Olive**

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Number of PhD students in the team: 7; number of M2 students in the team: 5; number of M1 students in the team: 3

Title of the project: **Immunotherapy & T cells: PD-1/CD28 interplay axis.**

Keywords: Immunotherapy, T cells, mouse models, flow cytometry

**Project summary:**

The outstanding clinical success of immune checkpoint blockade has revived the interest in underlying mechanisms of the immune system that are capable of eliminating tumors even in advanced stages. CD4 and CD8 T cells mainly control tumor outgrowth. Checkpoint blockade with programmed death-1 (PD-1) blocking antibodies induce tumor remissions and durable responses in cancer patients. A primary target for PD-1-mediated inhibition is the CD28 costimulatory molecule expressed at the T cell surface (1).

To further understand these mechanisms, we generated a genetic-modified mouse model where CD8 T cells are loosing the CD28 molecule (2) and expressing a TCR specific against a peptide expressed by melanoma cells (3), CD28 KO x pmel-1 TCR Tg mice).

The aim of this internship will be to characterize this mouse model by using PCR and Flow cytometry analysis. Then, *ex vivo* functional assays will be developed as cytotoxicity and cytokine production assays. In collaboration with the **TrGET Pre-clinical Assay Platform at CRCM**, tumor (melanoma) growth assays will be designed around a PD-1 immunotherapy using this mouse model.

In conclusion, this internship should give to the candidate, strong immunotherapy knowledge, inside a research team (35 people dedicated to human and mouse oncoimmunology into the IPC campus). The candidate will learn the basic techniques of immunomonitoring to further develop his/her career in this promising research field.

**References:**

1 - T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition.  

2 - In the absence of its cytosolic domain, the CD28 molecule still contributes to T cell activation.  

3 - Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance.  
Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): DNA Damage and genome instability
Name of the team/platform manager: Vincent Pagès
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Number of PhD students in the team: 0
Number of M2 students in the team: 0
Number of M1 students in the team: 1

Title of the project: Mutagenic or error-free bypass of DNA damages in vivo.
5 keywords: DNA damage, mutagenesis, DNA replication, homologous recombination

Project summary (1 page max):

During his Master 2 internship in our team, the student will use bacteria and yeast cells to studies the replication of a damaged DNA, and determine the genes and mechanisms involved in mutagenesis.

It has long been recognized that mutations in DNA are at the origin of genome instability, leading to several diseases among which cancer holds an important place. While mutations in the genome can arise spontaneously, they very often are the result of damages in the DNA. Indeed, the genome of all living organisms is constantly injured by endogenous and exogenous agents (UV light, cigarette smoke…) that modify the chemical integrity of DNA. Our overall goal is to understand how the cells deal with this damaged DNA. When damages are not repaired, cells possess two major strategies to tolerate residual lesions: i) translesion synthesis (TLS) where specialized DNA polymerases insert a few nucleotides opposite the lesion, with the possibility of introducing a mutation (Pagès & Fuchs 2002); ii) damage avoidance (DA) where the cells use homologous recombination to recover the genetic information from the sister chromatid, insuring survival in an error-free manner (Kuzminov 1999).

When dealing with accidental DNA lesions, mutagenic DNA damage tolerance mechanisms can lead to unwanted mutations, the initiating cause of cancer. On the other hand, when DNA...
damaging agents are used as therapeutics during chemotherapies, error-free tolerance mechanisms can lead to resistance to treatments. It is therefore essential to understand these mechanisms that encompass both TLS and DA.

In order to explore DNA damage tolerance, our team has developed an assay to monitor both error-prone (TLS) and error-free (DA) pathways simultaneously, by following the fate of a single replication-blocking lesion that we introduce in the genome of a living cell (Pagès & Fuchs 2018; Maslowska et al. 2019). Using this assay, the student will explore the genetics of lesion bypass and the structure of the replication fork that encounters a DNA lesion. More specifically, after inactivating or modifying specific genes, the studied lesion is inserted in the genome the cell (bacteria or yeast), and the bypass of the lesion is monitored by a colorimetric assay. We are routinely using a wide variety of molecular biology techniques (cloning, PCR, qPCR, Chromatin Immunoprecipitation, Next Generation Sequencing, etc…).

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team: **Cell Polarity, Signalling and Cancer**

Name of the team manager: **Jean-Paul BORG**

Supervisor name: **Marie-Josée SANTONI**
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Number of PhD students in the team: 4
Number of M2 students in the team: 1
Number of M1 students in the team: 0

Title of the project: **Study of Mechanism by which Lano/LRRC1 regulates cancer stem cell pool expansion**

5 keywords: **Cell polarity, Cancer stem cells, WNT secretion, Trafficking**

Project summary (1 page max):

Tumor initiation, progression and therapeutic resistance have been proposed to originate from a subset of tumor cells, cancer stem cells (CSC). However, the understanding of the mechanisms involved in their self-renewal and tumor initiation capacity remains limited. Stem cells and CSC self-renewal have been associated to alterations of NOTCH, Hedgehog, Hippo and WNT signaling, some of which have been causally linked to dysfunctional cell polarity. It is notably the case for SCRIB, which regulates planar cell polarity (PCP) through Hippo and β-catenin independent WNT signaling. SCRIB function has been extensively studied in mammary gland development and cancer progression using SCRIB-deficient or overexpressing mouse models. In breast cells, loss of SCRIB expression impairs directional cell migration and apico-basal cell polarity through the initiation of an EMT-like process concomitantly with the acquisition of CSC properties.

Our lab has cloned and characterized a mammalian SCRIB paralog, *Lano/LRRC1*, poorly study to date (Saito et al 2002). Recently, we have described its first function, Lano/LRRC1 expression is associated with a stem cell signature in normal and tumoral mammary epithelia. Through *in vitro* and *in vivo* experiments including a Lano/Lrrc1 knockout mouse model, we have demonstrated its involvement in the regulation of breast CSC fate and their pools expansion. Mechanistically, we have numerous clues demonstrating that Lano/LRRC1 suppresses WNT/β-catenin secretion and then related signaling induced in paracrine manner (Lopez Almeida et al., 2018).

The purpose of this project is to better define the mechanism by which Lano/LRRC1 negatively regulates WNT ligands secretion and thus the breast CSC pool.
Preliminary data have been obtained through a structure-function study trying to restore the initial rate of cancer stem cell population in Lano shRNA-depleted cells by ectopic expression of wild type Lano or its mutated versions. These results suggest that the Lano PDZ recognition sequence located at its C-terminal domain is required whereas other mutations, especially those in its N-terminus domain affecting its localization give a rescue comparable to the wild type control cells. Previously, Lano/LRRC1 partners involving its PDZ binding domain have been determined, through a yeast two hybrid screen comprising all human PDZ domains (Belloti et al., 2015). Among them some known to alter vesicle traffic, secretion and recycling have been identified and will be assessed for their role in mediating Lano/LRRC1 impact in breast CSC pool expansion and WNT ligands secretion. In a first step, the M2 student will confirm interaction between Lano/LRRC1 and its selected partners by biochemistry approaches. Then in a second step, she/he will study their effect on CSC pool expansion in our cell models measuring ALDH enzymatic activity and sphere formation efficiency as well as WNT ligands secretion through Elisa assays. Finally, in vivo, cancer cells are submitted to numerous environmental cues such as soluble factors as well as mechanical signals, thus she/he will test the combination of loss of expression of our proteins of interest with culture supports of diverse stiffness to measure the variation of their pool expansion in ours engineered breast cancer cell lines.

References:


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Cell polarity, Signaling and Cancer & MaP core facility
Name of the team/platform manager: Pr Jean-Paul Borg / Dr. Luc Camoin

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Number of PhD students in the team: 4
Number of M2 students in the team: 1
Number of M1 students in the team: 1

Title of the project: Active kinome related to LKB1/STRAD complexes

5 keywords: Quantitative mass spectroscopy, LKB1, STRAD, Active Kinome

Project summary (1 page max):

The incidence of lung tumors is growing, linked in particular to smoking and environmental factors. In 2012, in France, 40,000 new cases were identified, with a five-year survival of less than 15%. Lung cancer is a real public health issue.

With 80% of cases, non-small cell lung carcinomas (NSCLC) are by far the most common. Although, progress has been made it remain largely insufficient mainly because of the lack of diagnostic markers or therapeutic targets. Identification of tyrosine kinase dysregulation especially EGF-receptor activating mutations (EGF-R) or ALK-kinase-related chromosomal translocations led to the first targeted therapies (Cetuximab, Gefitinib and Crizotinib). However, these approaches only apply in a small number of cases (<7%) where these molecular events are found. In the case of NSCLC, the search for molecular events likely to be new prognostic markers or new therapeutic targets has brought to light that in more than 20% of the case, mutations of the STK11 gene encoding the serine/threonine kinase LKB1 (liver kinase B1). Generally associated with the expression of an oncogenic form of KRAS, these mutations lead to LKB1 catalytic activity loss. Hence, LKB1 kinase activity bears its tumor suppressor function.

To be triggered, LKB1 catalytic activity require its interaction with a STRAD pseudokinases (STRADa or its parologue STRADb) forming a functional complex. Both STRAD paralogues are expressed ubiquitously leading to co-existence in cells of both complex LKB/STRADa and LKB1/STRADb. These complexes have been characterized in vitro to exert a kinase kinase function since they can activate, by phosphorylation, 13 members of the subfamily of AMPK kinases (1). Although for a while LKB1 was mainly studied for its role in regulation of AMPK and associated consequences on the homeostasis of cellular metabolism, AMPK involvement in LKB1 tumor suppressor activity seems to be unrelated to energetic metabolism disorders commonly observed along tumor progression (2).

Through several approaches, including conditional knock-out mouse models, in vitro and in silico experiments, all indicate the complex encompassing the STRADb pseudokinase responsible for the LKB1 tumor suppressor activity relegating STRADa to a minor role. Since LKB1/STRAD complexes have a kinase kinase role and that tumors exhibit along tumorigenese dysregulated kinase activities, we propose for this Master 2 research project to...
define the "active kinome" related to each LKB1/STRAD complex and hence define the "active kinome signature" related to the LKB1/STRADb activity. This will contribute to decipher mechanism by which this complex exert its tumor suppressor properties but beyond "active kinome signature" related to the loss of this complex should also provide clues of dysregulated kinases potentially druggable as new targeted therapy for patients.

In this purpose cells invalidated for each STRAD including mouse embryonic fibroblast derived from their knock-out mouse models associated to expression of the oncogenic form of Ras will be used. Indeed, these cell models have shown a proliferative advantage in conditions deficient for STRADb suggesting these models recapitulate, at least in part, some of LKB1/STRADb tumor suppressor features. Active kinome of these cells will be defined by activity-based protein profiling using an ATP mimetic probe which desthiobiotinylate lysines near the catalytic active site allowing a subsequent avidin–biotin capture as previously described (3 & 4). Quantitative aspects will be carried by quantitative mass spectrometry using isobaric labeling for relative and absolute quantification (iTRAQ).

References:

1- Sebbagh M & al. The LKB1 complex-AMPK pathway: the tree that hides the forest. Fam cancer. 2011, 10(3) 415-424.


Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team/platform (please specify): Telomeres and chromatin
Name of the team/platform manager: Vincent Géli

Supervisor name: Marie-Noëlle Simon
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Number of PhD students in the team: 5
Number of M2 students in the team: 3
Number of M1 students in the team: 1

Title of the project: Spatial regulation of the replication fork repair pathways: implication for telomere stability and replicative senescence.

5 keywords: replication stress, telomere, DNA replication, homologous recombination, nuclear pore

Project summary (1 page max):

The ability of cells to survive and proliferate depends on the faithful and accurate duplication of the genome and failure in this process can cause mutations and genomic disorders. DNA replication is continuously threatened by a broad spectrum of exogenous DNA-damaging drugs but also unavoidable intrinsic replication fork barriers. Telomeres, at the ends of eukaryotic chromosomes, are part of these fragile sites that are a challenge for the replication machinery. Cells activate distinct DNA Damage Responses (DDR) to coordinate the signalling of stressed forks with their stability, repair and restart to avoid incomplete DNA replication, chromosome non-disjunction and telomere shortening. It recently became evident that certain types of DNA damage and designated DNA repair pathways are spatially localized in the nucleus. Telomeres are repeated sequences at the extremities of chromosomes that, together with a set of specific proteins, protect terminal ends against degradation and fusion. Telomere length is maintained by a specific reverse transcriptase called telomerase. Upon inactivation of telomerase, telomeres get shorter at each replication cycle. When telomeres get too short, cells enter replicative senescence and stop proliferating. Replication lesions can be “repaired” by the telomerase but when telomerase is not produced, as in most somatic cells, repair of replication fork stalled at telomeres depends on homologous recombination.
We, and others have shown in the model yeast *Saccharomyces cerevisiae* that the Nuclear Pore Complex (NPC) determine the pathway used to repair some DNA damage such as double strand breaks or very short telomeres\(^3\)\(^4\). Our most recent data show that NPCs are also instrumental for conservative, recombination-dependent repair of replication lesions at telomeres in the absence of telomerase\(^5\).

Using the power of yeast genetics associated with molecular biology and cell biology approaches, the student will investigate the molecular determinants responsible for relocalization to the NPC of the replication fork stalled at telomeres. He/she will analyze the functional consequences of relocalization defects on fork repair, telomere maintenance and senescence. Recent publications suggest that spatial regulation and the role of NPC in the DNA damage response are conserved in metazoans. The results from this study will be of general interest for a broad community working on replication stress and genome stability.

References:


Master 2 Project title: METABOLIC TARGETING OF METASTATIC PANCREATIC CANCER

CRCM Group: Metabolism of pancreatic cancer
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Concept and Objectives : Pancreatic ductal adenocarcinoma (PDAC) is the most intractable solid cancer with a 5-year survival below 8%. More than 80% of patients with PDAC are diagnosed with an unresectable tumor and are therefore not eligible for surgery. PDAC cells reprogram their metabolism to survive in their harsh hypoxic and nutrient deprived microenvironment, and this metabolic shift favors the survival of the most aggressive cells and their dissemination at distant sites. The metabolic changes associated with the metastatic progression of the PDAC remain crucial opened-questions. Microenvironment composition (cellular/acellular components and nutrient availability) differ tremendously in the primary and metastatic sites (liver and/or lung). Hence, cancer cells must develop a metabolic flexibility to satisfy their biomass and energetic demands required for their proliferation and expansion in both primary and metastatic sites. In this project, we aim at identifying and targeting the metabolic pathways that are activated i/ in aggressive mesenchymal tumour cells, i.e. those endowed with metastatic features because undergoing through the epithelial-to-mesenchymal transition (EMT) at the primary tumor site, and ii/ in liver metastases. We will also proceed to a robust clinical validation of selected metabolic targets using samples from patients resected PDAC.

We have already identified the transcriptomic metabolic signatures of mouse PDAC and of its matched liver metastases. Amino-acids related metabolic pathways, and especially the methionine pathway, are activated in metastasis compared to the primary tumors and interestingly are also activated in EMT PDAC cells. We have demonstrated that blocking the methionine pathway limits PDAC growth and the migratory potential of tumor cells. For the Master 2 training we aim at : 1/ defining the metabolic enzymes of the methionine pathway participating to the acquisition of an aggressive EMT phenotype leading to the emerging metastatic tumor cells, 2/ targeting by genetic silencing and pharmacological inhibition enzymes driving the tumor cells metastatic features and evaluate functional consequences of this targeting in vitro and on pre-clinical mouse models for relevant enzymes.

Methods : in vitro : use of pancreatic tumor cell lines, technics of molecular and cellular biology (Western-Blot, RT-qPCR, functional read-out : migration, proliferation, cell death, FACS). In vivo : use of PDAC samples from a genetic engineered mouse model developing spontaneously PDAC in order to validate localization in PDAC of the metabolic enzymes chosen as candidates. Use of orthotopically induced PDAC mouse models for pre-clinical validation of the silencing of methionine enzymes.

References :