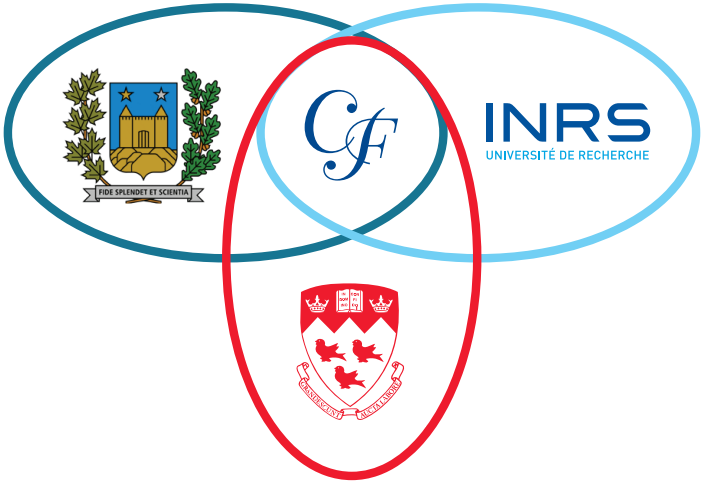
Cole Foundation
"Research Celebration Day"

May 13, 2016

10th Year



10ième Année

Journée
« Célébrons la recherche »
de la Fondation Cole

13 mai 2016

The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD programmes to promote research in pre-leukemia, leukemia, and other leukemia-related diseases in children and young adults, as well as the development of clinical care for patients affected by these diseases. With the announcement of 2016 Fellows, the Fellowship programme has supported more than 150 researchers in laboratories and hospitals situated in the Greater Montreal area through collaborations with l'Université de Montréal, McGill University, and INRS - Institut Armand-Frappier Research Centre. Over $10 million has been committed to this programme.

The Cole Foundation was established in 1980 by John N. (Jack) Cole as a private family foundation to provide funds for medical facilities concerned with child care and research towards a cure for leukemia. The Foundation also awards grants to worthy community causes. The catalyst for the Foundation's creation was the death of his daughter, Penny Cole, from leukemia over a decade earlier.

Through the Foundation, Jack Cole provided millions of dollars for medical facilities in Montreal. Notable among his achievements was the support of the Penny Cole Laboratory at the Montreal Children's Hospital and the establishment of the Jack Cole Chair in Pediatric Oncology and Hematology at McGill University.

Since his death in 2004, the Foundation has continued to carry on Jack Cole's mission with a renewed focus on research and child care for young patients afflicted with leukemia and leukemia-related diseases.

La Fondation Cole

La Fondation Cole soutient la recherche sur le syndrome myélodysplasique, la leucémie et d'autres affections liées à la leucémie chez les enfants et les jeunes adultes, ainsi que le développement des soins cliniques pour les personnes atteintes de ces maladies, en offrant des bourses à des chercheurs-cliniciens, des résidents postdoctoraux et des étudiants diplômés inscrits à des programmes de doctorat. Avec l'annonce des boursiers 2016, le programme a appuyé plus de 150 chercheurs répartis dans des laboratoires et des hôpitaux de l'agglomération montréalaise, à la faveur de collaborations avec l'Université de Montréal, l'Université McGill et le Centre INRS – Institut Armand-Frappier. Plus de dix millions de dollars y ont été consacrés.

La Fondation Cole a été instituée en 1980 par John N. (Jack) Cole à titre de fondation familiale privée pour subventionner des services de santé s'intéressant aux soins et à la recherche en pédiatrie et à la découverte d'un traitement curatif pour la leucémie. Elle a été créée à la mémoire de sa fille, Penny Cole, emportée par la leucémie plus de dix ans avant. La Fondation appuie aussi des causes communautaires louables.

Grâce à la Fondation, Jack Cole a donné des millions de dollars à des services de santé de Montréal. Parmi ses grandes réalisations figurent son soutien du Laboratoire Penny Cole à l'Hôpital de Montréal pour enfants et la création de la chaire Jack Cole en oncologie-hématologie pédiatrique à l'Université McGill.

Depuis le décès de son maître d'oeuvre en 2004, la Fondation poursuit sa mission avec un intérêt marqué pour la recherche et les soins pédiatriques destinés à de jeunes patients affectés par la leucémie ou une maladie liée à la leucémie.

Program

9:30 - 10:30 am	Round Table (new fellows) Holmes Hall, 3605 rue de la Montagne
10:00 - 11:00 am	Poster Session Part 1
11:00 - 12:00 pm	Poster Session Part 2
12:00 - 12:45 pm	Lunch/Dîner
1:00 - 2:00 pm	Lecture *Martin Amphitheater, McIntyre Building*
Welcome	*Dr. Shari Baum* *Associate Dean, Research,* *James McGill Professor, McGill*
New Research - New Realities Presentations	*Dr. Noel Raynal* *Hôpital Sainte Justine* *"Drug Repositioning Approach for Epigenetic Therapy for Pediatric Acute Leukemia"*
	Dr. Moutih Rafei *Université de Montréal* *"The Hidden Side of Interleukin-21"*
	Dr. Geraldine Delbes *Institut Armand-Frappier* *"Optimizing fertility preservation in boys with cancer"*
2:00 - 3:15 pm *Introduction*	Dr. Kolja Eppert *Assistant Professor, the Department of Pediatrics, McGill University*
Keynote Speaker	*Dr. Stephen Hunger* *Chief of the Division of Oncology and the Director of the Center for Childhood Cancer Research at The Children's Hospital of Philadelphia, PA.* **"Developing Precision Medicine Therapies in Pediatric Acute Lymphoblastic Leukemia"**
3:15 - 3:30 pm	New fellows and announcement of prizes
3:30pm	Reception

The 2016 - 2018 Cole Foundation Fellows
Les boursiers de recherche de la Fondation Cole 2016 – 2018

McGill University

Hosni Cherif, Post PhD program
Supervisor: Alexandre Orthwein, Lady Davis Institute
Project title: Identification of novels targets and biomarkers for the treatment and the diagnosis of the GCB-DLBCL pediatric lymphoma
Description: Lymphomas constitute ~8% of all pediatric cancer cases. One of the most common and aggressive lymphomas is Diffuse Large B-cell Lymphoma (DLBCL). The aim of my project is to identify novel biomarkers to predict the risk for DLBCL to resist to current therapies and identify novel targets for the treatment of this malignancy.

Heather Duncan, PhD program
Supervisor: Kolja Eppert, McGill University Health Centre, Pediatric Hematology-Oncology
Project title: G protein-coupled receptor 56 as a potential regulator of leukemic stem cells
Description: Leukemic stem cells sustain acute myeloid leukemia and resist chemotherapy leading to poor outcome in patients. We identified G-protein coupled receptors (GPCRs) with increased expression in stem cells that correlate with poor overall survival. This project investigates the function of GPCRs in leukemic stem cells in vitro and in vivo.

Deanna MacNeil, PhD program
Supervisor: Chantal Autexier, Lady Davis Institute
Project title: Telomeras regulation through post-translational modifications of dyskerin
Description: This project focuses on developing a more comprehensive understanding of the telomerase protein dyskerin and its regulation, which is needed to develop prevention and treatment strategies targeting telomerase-related health issues like bone marrow failure syndromes and cancers of the blood.

Claudia Wever, Post PhD program
Supervisor: Nathalie Johnson, Lady Davis Institute
Project title: Understanding apoptotic blocks in primary non-Hodgkin lymphoma samples
Description: I use a technique called BH3 profiling on lymphomas to uncover obstructions in programmed cell death pathways. With this information, we can choose drugs that specifically target the proteins that are keeping the cells alive, and therefore personalize treatments based on the characteristics of each person's cancer.

Ke Zhi Yan, PhD program
Supervisor: Xiang-Jiao Yang, Rosalind and Morris Goodman Cancer Center
Project title: Role of the epigenetic regulator BRPF1 in leukemia development
Description: Aberrant fusion proteins containing histone acetyltransferase called MOZ promote leukemia development. Such fusion proteins form complexes with a novel epigenetic regulator BRPF1. My research is to investigate how BRPF1 may regulate the ability of MOZ-containing fusion proteins to induce leukemia and confer leukemic stem cell properties to differentiated blood cells.

Université de Montréal

Rachid Abaji, PhD program
Supervisor: Maja Krajinovic, CHU Centre de recherche Sainte-Justine
Project title: Using whole exome sequencing data to identify pharmacogenetics markers in childhood leukemia
Description: Investigating the influence of whole exome sequencing- derived genetic variants on the outcome of Acute Lymphoblastic Leukemia in children. The functional impact of validated significant variants is then assessed for further information that can be integrated into clinical practice to guide dosing regimens towards more effective treatments with minimum toxicity.

Léo Aubert, Post PhD program
Supervisor: Philippe Roux, IRIC, Pathology and Cell Biology
Project title: Characterization of the surfaceome of normal and leukemic stem cell expanded with UM171 to improve the development of novel therapies for leukemia
Description: This collaborative project is part of a research program that aims to improve our knowledge of the molecular mechanisms governing HSCs and LSCs self-renewal, with the ultimate goal of helping patients with leukemic disorders through the optimization of cord blood grafts and the development of new targeted therapies.

Yahya Benslimane, PhD program
Supervisor: Léa Harrington, IRIC, Medicine
Project title: Investigation synthetic sensitivity to telomerase inhibition in leukemia
Description: We will use genome-wide screens to determine which gene functions whose depletion sensitizes acute lymphoblastic leukemic (ALL) cells to telomerase inhibition. These gene networks will advance our understanding of how cells rely on different modes of telomere maintenance for survival, and will also potentially identify new therapeutic strategies for ALL.

Aubrée Boulet-Craig, PhD program
Supervisor: Sarah Lippé, CHU Centre de recherche Sainte-Justine, Neuropsychologie
Project title: Corrélats neuroanatomiques et neurofonctionnels prédisposant à l›apparition de difficultés en mathématiques chez des survivants de leucémie lymphoblastique aiguë pédiatrique
Description: Les survivants de leucémie lymphoblastique aiguë (LLA) pédiatrique présentent des difficultés en mathématiques et en mémoire à court terme (MCT). L'objectif du projet est de vérifier si les troubles de la MCT sur le plan comportemental, neuronal et neuroanatomique prédisent les difficultés en mathématiques chez les survivants LLA.

Willow Burns, PhD program
Supervisors: Serge Sultan, Sainte-Justine Hospital Research Center, Université de Montréal, Psychology & Pediatrics;
Katherine Péloquin, Université de Montréal, Psychology
Project title: Towards a better understanding of the adjustment of parents facing childhood leukemia: Exploration of dyadic for acting at the family level
Description: Since pediatric ALL patients' distress is intricately intertwined and dependent on the distress of their primary caregivers (i.e., parents - individually and as a parental couple), we must also attend to the needs of the family. Through dyadic analyses this research explores interdependent patterns of relationship adjustment for both partners.

Margaret Davis, Post PhD program
Supervisors: Katherine Borden, IRIC, Pathology and Cell Biology
Project title: Novel functions of eukaryotic translation initiation factor 4E (eIF4E) in leukemia
Description: This project aims to define the novel role of eukaryotic translation initiation factor 4E (eIF4E) in 3' end processing of RNA. In addition, this study will investigate the extent of this function in adult and pediatric leukemia and lymphoma.

Marion Dubuissez, Post PhD program
Supervisors: Éric Milot, Maisonneuve-Rosemont Hospital
Project title: Rôles du facteur de transcription Ikaros dans la terminaison de la transcription
Description: La mutation/délétion du suppresseur tumoral Ikaros est associée au développement des LLAs pédiatriques de mauvais pronostic. Dans différents cancers, la terminaison anormale de la transcription a été détectée et module l'expression génique. Nous étudions le rôle du facteur de transcription Ikaros dans la terminaison de la transcription lors de l'hématopoïèse.

Fida Khater, Post PhD program
Supervisors: Daniel Sinnett, CHU Centre de recherche Sainte-Justine, Hemato-Oncology
Project title: Identification et validation fonctionnelle de nouvelles cibles thérapeutiques chez les patients leucémiques en rechute dans le cadre de l›étude de faisabilité TRICEPS
Description: Mon projet de recherche s'insère dans l'étude de faisabilité TRICEPS lancée en avril 2014, et qui vise à caractériser le profil mutationnel tumoral pour chaque patient réfractaire au traitement ou en rechute recruté pour cette étude, via un séquençage intégratif (génomique et transcriptomique) du matériel tumoral et de sa contrepartie normale dans le but de proposer une stratégie thérapeutique ciblée et personnalisée.

Céline Laumont, PhD program
Supervisors: Claude Perrault, IRIC, Immunobiology
Project title: Identification of leukemia-specific antigens: a k-mer profiling approach
Description: Despite evidence supporting their clinical relevance in T-cell based immunotherapy, the molecular structure of leukemia-specific antigens (LSAs) is still unknown. Our novel approach combining RNA-sequencing and mass spectrometry will enable us to reliably identify those LSAs that can then be used for the treatment of leukemias.

Vincent-Philippe Lavallée, Clinician program
Supervisors: Guy Sauvageau, IRIC, Medecine
Project title: Towards an optimized prognostic classification of AML patients using novel markers, and improvement in diagnosis and treatment of acute promyelocytic leukemia related coagulopathy
Description: In my project, I propose to directly improve diagnostic, prognostication and treatment of AML patients by clinical validation of novel prognostic markers in AML. We have recently identified 2 very promising new prognostic markers in AML patients and preliminary data indicates a high expression of these 2 genes in a large group of patients that have an adverse clinical outcome. In addition, I plan an analysis of "gene A" role in APL-related coagulopathy, and determination of its utility as a novel diagnostic marker and therapeutic target. I have recently identified a novel gene that appears to be a key player in APL-related coagulopathy. I will characterize the role of this gene in platelet aggregation and coagulation *in vitro* and *in vivo*. In addition, I will assess its utility as a diagnostic marker for APL and determine whether protein blockade using small molecules or antibodies could treat or prevent APL-related coagulation disorders.

Charles-Etienne Lebert-Ghali, Post PhD program
Supervisors: Heather Melichar, Maisonneuve-Rosemont Hospital
Project title: The role of CD271 in the suppression of graft-versus-host-disease by mesenchymal stem cells
Description: Mesenchymal stromal cells suppress T cell function and are being used with some success to treat graft-versus-host-disease (GVHD) in pediatric leukemia patients who underwent bone marrow transplantion. We are defining the role of a novel immunomodulatory protein on these cells to enable more effective therapeutic strategies against GVHD.

Sophia Morel, PhD program
Supervisors: Valérie Marcil, CHU Centre de recherche Sainte-Justine, Nutrition
Project title: Gut microbiota and cardiometabolic complications in survivors with childhood acute lymphoblastic leukemia
Description: This research project aims at studying if modifications in intestinal bacteria and in gut-related metabolites could explain the development of the cardiometabolic disruptions observed in survivors of childhood acute lymphoblastic leukemia.

Nandita Noronha, PhD program
Supervisors: Guy Sauvageau, IRIC, Medecine
Project title: Validation of the HOX-MEIS1-PBX oncogenic complex as a bona fide therapeutic target in primary human leukemias
Description: The HOX-MEIS-PBX (HMP) complex is one of the most commonly deregulated molecular network in leukemia patients. Our project aims at characterizing the role of each of the components of this protein complex in leukemogenesis with the hope of identifying novel biological targets for this disease.

Pierre Priam, PhD program
Supervisors: Guy Sauvageau, IRIC, Chromatin Structure and Stem Cell Biology
Project title: Role of SMARCD2, SWI/SNF subunit, in leukemia maintenance

Description: Recent studies indicate that the SMARCD2 subunit of SWI/SNF chromatin remodeling complexes is essential for leukemic cell proliferation. The goal of this project is to provide a validation of SMARCD2 dependency and mechanism of action in human AML for the eventual identification of specific inhibitors in leukemia treatment.

Christina Sawchyn, PhD program
Supervisors: Frédérick A. Mallette, Maisonneuve-Rosemont Hospital
Project title: Characterization of the KDM4A histone demethylase in pediatric acute myeloid leukemia
Description: The goal of this project is to establish the precise role of a chromatin regulator, KDM4A, for the maintenance of leukemic cells. Focus will be placed identifying yet unknown genomic targets of KDM4A and characterizing its chemical inhibition as a target for novel therapies.

Aditi Sood, Post PhD program
Supervisors: Heather Melichar, Maisonneuve-Rosemont Hospital
Project title: Optimizing the T cell repertoire for T-cell therapy for leukemia
Description: T cell therapy has emerged as a promising treatment for pediatric leukemia. However, a major challenge to durable patient responses is a lack of T cell persistence and function in vivo. We aim to identify T lymphocyte subsets that will overcome these hurdles to improve the efficacy of this therapy.

Cole Foundation Poster Session
Session d'affiches de la Fondation Cole

Presenters / Liste des exposants

Haithem Barbour
Karine Bourdage
Marina Bury
Sophie Cardin
Xi-Lin Chen
Nadine Sen Nkwe Dibondo
Michael Forster
Qianyu Guo
Said Izreig
David Kachaner
Vincent-Philippe Lavallée
Valérie Lemay
Ludivine Litzler
Rayelle Itoua Maiga
Adam-Nicolas Pelletier
Alexandre Rouette
Camille Simon
Clarisse Thiollier
Elisa Tomellini
Aurelie Tormo
Diogo Veiga
Peng Wang

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P04	Cardin, Sophie	Modeling of pediatric acute megakaryoblastic leukemia using cord blood/stem progenitor cells
P05	Chen, Xi-Lin	eIF4E3 inhibits eIF4E mRNA export and oncogenic potential correlating with modulation of its subcellular trafficking
P06	Dibondo, Nadine Sen Nkwe	Role of USP16 coordinating the DNA Damage Response
P07	Forster, Michael	Mysm1 as a potential drug target for p53 activation in B cell malignancies
P08	Guo, Qianyu	Mnk1 promotes in the progression of breast ductal carcinoma in situ to invasive ductal carcinoma
P09	Izreig, Said	miR-17~92 is a global metabolic regulator in Myc-driven lymphoma

P10	Kachaner, David	Molecular mechanisms of spatio-temporal regulation of the Polo kinase in cell division
P11	Lavalléee, Vincent-Philippe	Chemo-genomic interrogation of primary acute myeloid leukemia with biallelic *CEBPA* mutations reveals recurrent *CSF3R* mutations and subgroup sensitivity to JAK inhibitors
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P13	Litzler, Ludivine	PRMT5 regulates B cell development and germinal center homeostasis
P14	Maiga, Rayelle Itoua	Structure/Function Analysis of the c-MYC Proto-oncogene mRNA
P15	Pelletier, Adam-Nicolas	Characterization of RET in MLL-AF9 acute myeloid leukemia
P16	Rouette, Alexandre	Expression of immunoproteasome genes is regulated by cell-intrinsic and –extrinsic factors in human cancers
P17	Simon, Camille	Ezh1 and Ezh2 are essential regulators of normal and leukemic hematopoietic stem cells
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P19	Tomellini, Elisa	A chemo-genomics approach to dissect and increase self-renewal divisions of hematopietic stem cells
P20	Tormo, Aurelie	Interleukin-21 accelerates T-cell reconstitution following allogeneic bone marrow transplantation or human cord blood transplantation
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P01

Title: Regulation of the leukemia suppressor TET2 by OGT and CXXC1

Authors: Haithem Barbour, PhD 2014-2016

Affiliation: Haider H. Dar, Salima Daou, Jessica Gagnon, and El Bachir Affar, Centre de Recherche Hôpital Maisonneuve-Rosemont, Université de Montréal, Canada.

Keywords: Leukemia, TET2, 5hmC, OGT, CXXC1

Background information: TET2 is one of the most mutated tumor suppressor genes in hematological malignancies including pediatric acute-myeloid leukemia (AML). TET2 has a unique enzymatic activity in mammalian cells, in fact, it transforms methylated cytosine (5mC) repressive DNA mark to hydroxyl-methyl-cytosine (5hmC) which promotes the activation of target-gene transcription. In leukemia, multiple TET2 loss-of-function mutations have been reported. Nevertheless, our understanding of the effect of these mutations on TET2 activity and on its interaction with other proteins remains largely elusive.

Purpose of the study: Our aim is, on one hand, to understand the dynamics of TET2's interaction with OGT in normal and leukemic cells. On the other hand, we aimed to unveil the mechanism by which TET2 is recruited to DNA. We also aimed to identify the impact of defects in TET2 recruitment and activity in leukemic transformation.

Methods: To fulfill our goals, we used multiple methods including protein Tandem-Affinity Purification (TAP) to purify the TET2-OGT complexes which were subjected to mass-spectrometry analysis to identify the interacting partners. We also implemented multiple bioinformatics strategies to analyze TET2 expression and predict its target genes.

Results: Our results show that TET2 is indeed glycosylated by OGT. We have purified the glycosylated form of TET2 which we subjected to Mass Spectrometry analysis and identified the glycosylation sites on TET2. We show also that, when expressed together, OGT inhibits TET2 catalytic activity. Strikingly, by purifying of TET2 complex have identified CXXC1 as a new interacting partner of TET2. In fact our results show that CXXC1 interacts with TET2 in vivo and increases its stability and catalytic activity towards 5mC. Interestingly, we also found that CXXC1 promotes the recruitment of TET2 to the chromatin. Since the interaction with CXXC1 affects the stability and recruitment of TET2 we wanted to investigate the expression level of TET2 and its interacting partners in cancer cell lines. Our results of RNA-Seq analysis suggest that both TET2 and OGT are amplified in AML patients. Intriguingly, CXXC1 does not seem to be amplified suggesting a more complex mechanism by which TET2 is regulated on both the mRNA and protein levels. Furthermore, we used a bioinformatics approach to identify TET2 direct and indirect target genes in cancer cell lines in general and in AML cells in particular and we found 37 genes which positively correlate with TET2 expression levels specifically in AML including multiple transcription factors.

Conclusion: TET2 is a critical tumor suppressor highly mutated in hematological malignancies and our study shed more light on how TET2 is regulated on both mRNA levels and protein levels in AML cells and other cancer types. Also we identified how TET2 catalytic activity towards 5mC is regulated by TET2's major partners OGT. TET2 is a member of TET family which lacks a DNA binding domain and our study has identified CXXC1 as a new linker between DNA and TET2.

P02

Title: Deciphering Fbw7-specific susceptibilities in cancer using a genome-wide CRISPR approach

Author: Karine Bourdages, PhD 2015-2017

Affiliation: Thierry Bertomeu, Jasmin Coulombe-Huntingdon, Mike Tyers, Department of medicine, Université de Montréal, Institut for Research in Immunology and Cancer (IRIC)

Keywords: Acute lymphoblastic leukemia, Fbw7, CRISPR, genome-wide, synthetic lethal

Background information: The tumor suppressor Fbw7 is one of the most frequently mutated genes in cancers. More specifically, Fbw7 is mutated in 31% of T-cell acute lymphoblastic leukemia. Fbw7 ubiquitin ligase targets hundreds of substrates for degradation, including the oncogenes MYC, JUN and $Notch^{ICD}$. Loss of Fbw7 function thus leads to increased proliferation and genome instability.

Purpose of the study: We hypothesize that cancer cells mutated for Fbw7 may be vulnerable to loss of other gene functions, termed synthetic lethality. We are undertaking systematic Fbw7+/- synthetic lethal screens in T-ALL and other blood cancer types cells lines in order to uncover potential new therapeutic targets.

Methods: To uncover upstream regulators and downstream effectors of Fbw7 and to identify Fbw7-specific susceptibilities in cancer, large-scale CRISPR screens are undertaken. We acquired custom pools of sgRNAs covering the entire human genome that were cloned into lentiviral vectors for infection of Cas9 cell lines either wild type or mutated for Fbw7.

Results: We already performed a whole-genome knockout screen in a human pre-B ALL cell line wild type for Fbw7. Data from this screen will be compared to those we obtain from other screens in cell lines mutated for Fbw7. Amongst those, Jurkat cells mutated for Fbw7 are currently screened. We expect to observe gene deletions that affect Jurkat (Fbw7+/-) cell viability to be depleted from the pool compared to Fbw7 wild type lines, such as our previously screened pre-B ALL cell line. Those will be assessed by next generation sequencing and thereby identify genes that cause lethality in combination with Fbw7 mutations.

Conclusion: We expect to identify a robust set of genes that cause Fbw7-specific synthetic lethality, which could represent novel targets for the future design of small molecule inhibitors. We are optimistic that this project will help identify new therapeutic targets for cancers bearing mutations in Fbw7.

P03

Title: Regulation of NFE2L3 (NRF3) in hematopoietic cells: linking transcription factor function to lymphomagenesis

Authors: Marina Bury, Post PhD 2015-2017

Affiliation: Joo Yeoun Park, Yusra K. Al Mosuli, Anna Derjuga, Volker Blank, Dr. Volker Blank, Experimental Medicine, Lady Davis Institute

Keywords: lymphoma, hematopoiesis, NFE2L3

Background information: NFE2L3 (nuclear factor, erythroid 2-like 3 or NRF3) is a member of cap'n'collar (CNC) transcription factors that play key roles in mammalian gene regulation, stress response and oncogenesis. This basic leucine zipper protein is stringently regulated with a high turnover. Different versions of the protein are located in nucleus, cytosol and ER, indicating complex intracellular regulation. NFE2L3 interacts with small Maf proteins to mediate cellular detoxification and stress response signaling. We further found high NFE2L3 transcript levels in the thymus. Our *in vivo* studies showed that *Nfe2l3*$^{-/-}$ mice exhibit higher morbidity, mortality as well as susceptibility to T-cell lymphoblastic lymphoma upon the exposure to carcinogen benzo[a] pyrene (B[a]P). Our data suggest that NFE2L3 has a protective role in carcinogen-induced lymphomagenesis.

Purpose of the study: Although our previous data linked NRF3 to lymphomagenesis, its mechanism of action has not been analyzed in detail. We, thus, propose to elucidate the molecular mechanisms governing the regulation of NFE2L3 in hematopoietic cells as well as its role in lymphomagenesis.

Methods: We performed FACS and complete blood count (CBC) studies to respectively analyze hematopoietic stem cells (HSCs), progenitor cells and fully differentiated hematopoietic cells in *Nfe2l3*$^{-/-}$ mice. To gain insights into the regulation of NFE2L3, we treated Jurkat and Namalwa lymphoma cell lines with tumor necrosis factor-alpha (TNFa) and lipopolysaccharide (LPS). In addition, we performed loss-of-function studies using shRNA specific for NFE2L3 and examined the proliferation of these cells by a colorimetric MTT viability assay. Furthermore, in collaboration with Dr. Gascoyne's laboratory (UBC), we identified three somatic mutations in diffuse large B-cell lymphoma patient samples, using RNA-sequencing. We performed luciferase reporter assays to evaluate the transactivation activity of these NFE2L3 mutants.

Results: The absence of NFE2L3 resulted in major changes in hematopoietic cell fate, as we observed a significant decrease in the long term-hematopoietic stem cells (LT-HSCs) and common lymphoid progenitors (CLPs) as well as a considerable increase in multipotent progenitors (MPPs). In contrast, CBC and FACS data showed no significant difference in terms of fully differentiated blood cells. In our cell culture studies, we found that the protein level of NFE2L3 is induced by TNFa and LPS in both Jurkat and Namalwa cells. Exposure to TNFa also led to increased phosphorylation of IkBa. Using a MTT assay, we further showed reduced proliferation of Namalwa cells upon NFE2L3 knockdown. Of interest, NFE2L3 mutants, harboring three mutations, S481N, D644G or N346K, identified in patients with diffuse large B-cell lymphoma, showed a significant decrease in transactivation activity in luciferase reporter assays

Conclusion: In conclusion, our recent *in vivo* data suggest that NFE2L3 significantly alters the differentiation and growth of hematopoietic cells, controlling cell fate decisions. Our cell culture data suggest that NFE2L3 plays a role in the intracellular signaling of lymphoma cells and that its expression is controlled by the NFkB pathway. In addition, mutations in NFE2L3 identified in large B-cell lymphoma patients may result in a transcription factor with reduced ability to transactivate. Together, our data suggest crucial functions of NFE2L3 in hematopoietic cells. Further investigation into the role of NFE2L3 will provide valuable insights for the treatment of hematological malignancies.

P04 *(Absent)*

Title: Modeling of pediatric acute megakaryoblastic leukemia using cord blood/stem progenitor cells

Author: Sophie Cardin, Post PhD 2015-2017

Affiliation: Sonia Cellot, Université de Montréal, CHU Sainte-Justine.

Keywords: NUP98-KDM5A fusion, acute megakaryoblastic leukemia, cord blood stem/progenitor cells

Background information: Acute megakaryoblastic leukemia (AMKL) is a subset of pediatric acute myeloid leukemia associated with poor outcome (5-year overall survival <50%) and molecular heterogeneity. A fusion between the genes encoding for the nucleoporin NUP98 and the histone demethylase KDM5A (NUP98-KDM5A fusion) is reported as a recurrent mutation in 10-15% of AMKL. Modelling of AMKL in the physiologically relevant context of human cells is critically warranted to identify leukemia specific targets and biomarkers.

Purpose of the study: We developed *in vitro* and *in vivo* models of human AMKL through overexpression of NUP98-KDM5A in cord blood hematopoietic stem/progenitor cells (CB-HSPC).

Methods: Using lentiviral transduction and optimized culture conditions for CB-HSPC, the NUP98-KM5A fusion was overexpressed in $CD34^+$ cells isolated from cord blood units. The GFP reporter gene of the lentiviral construct enabled tracking of the transduced CB-HSPC (GFP^+ cells). Fractions of independently seeded cell cultures were transplanted into immunodeficent mice or expanded *in vitro*. Cell cultures were analyzed at regular intervals for GFP content, cytology, immunophenotype and clonogenic activity. Expression profiling of CB-HSPC, NUP98-KDM5A overexpressing cells and pediatric AMKL samples was performed using RNAseq.

Results: *In vitro*, NUP98-KDM5A-transduced HSPC displayed marked enrichment for GFP^+ cells (~5% to >90%) in 2 independent cultures after 90 days. The emergence of this GFP^+ cell population was associated with maturation arrest, increased progenitor frequency (30% vs 1%) and immunophenotype ($CD34^+$, $CD71^+$, $CD96^+$) suggestive of leukemic blasts, vs control cells (100% mast cells). Expression profiling of the 2 cell lines unravelled de-repression of the 5'HOXB gene cluster, as well as other transcription factors and epigenetic regulators similar to NUP98-KDM5A AMKL patient samples. *In vivo*, NUP98-KDM5A-transduced HSPC (day8 of culture) gave rise to a myeloproliferative disorder that mirrors pediatric AMKL phenotype. Epigenetic/genomic studies as well as functional confirmatory assays are ongoing.

Conclusions: Collectively, these results demonstrate that overexpression of NUP98-KDM5A in CB-HSCP can lead to leukemia development *in vivo* and to establishment of cell lines *in vitro*.

This model can be exploited to identify leukemia specific targets and biomarkers (vs CB-HSPC and patient AMKL samples). The characterized cell lines will be used for biochemical studies and chemical/genetic screens.

P05

Title: eIF4E3 inhibits eIF4E mRNA export and oncogenic potential correlating with modulation of its subcellular trafficking

Authors: Xi Lin Chen, Post PhD 2015-2017

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Keywords: Eukaryotic translation initiation factor, mRNA export, Acute myeloid leukemia, Nuclear trafficking, Oncogenic potential

Background information: Acute myeloid leukemia (AML) is an aggressive malignancy characterized by accumulation of immature myeloid cells in the bone marrow. The mRNA cap-binding protein eukaryotic translation initiation factor 4E (eIF4E) is an oncogene that regulates gene expression post-transcriptionally at multiple levels including mRNA translation and mRNA export, which is involved in proliferation, survival and oncogenic transformation. eIF4E is substantially elevated and forms abnormally large nuclear bodies in the M4/M5 subtypes of AML primary specimens. Conversely, eIF4E3, a novel eIF4E family member, suppresses eIF4E dependent nuclear export of target mRNA and oncogenic transformation. Further, in M4/M5 AML patient specimens, eIF4E3 expression is severely reduced. However, the mechanisms of how eIF4E3 counteract eIF4E are still unknown.

Purpose of the study: Investigate whether eIF4E3 can suppress oncogenic eIF4E activity by inhibiting its mRNA export via modulating eIF4E's subcellular trafficking.

Methods: U2OS cells were stably transfected with plasmids encoding either eIF4E or eIF4E3 or both. By using western blot, the expression of eIF4E target proteins was examined. Cells were fractionated into nuclear and cytoplasmic components and mRNA levels in each fraction were investigated using qPCR. Immunofluorescence in conjunction with confocal laser microscopy was performed to determine the localization of eIF4E in U2Os cells. Finally, an anchorage-dependent foci formation assay was used to determine how eIF4E3 affects the oncogenic potential of eIF4E.

Results: The expression of established eIF4E targets NBS1, c-Myc and Mcl-1 protein levels were reduced by eIF4E3 overexpression relative to vector control and eIF4E overexpressing cells. Accordingly, eIF4E3 overexpression led to nuclear accumulation of eIF4E mRNA export targets such as NBS1, c-Myc and Mcl-1 without altering their total mRNA levels, which is consistent with their reduced protein expression. Next, the overexpression of eIF4E3 moved much of the nuclear localized endogenous and exogenous eIF4E to the cytoplasm and the nuclear staining of eIF4E is reduced. Furthermore, we showed that eIF4E leads to increased cell foci number and this was reversed by eIF4E3 overexpression, which related to reduced oncogenic activity.

Conclusion: Taken together, eIF4E3 inhibits oncogenic eIF4E mRNA export activity by retaining eIF4E in the cytoplasm. eIF4E3 may also compete for eIF4E target RNAs and sequester them. These activities likely underlie its ability to repress eIF4E's oncogenic potential in AML.

P06

Title: Role of UAP16 in coordinating the DNA damage response

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Keywords: Leukemia, Ubiquitination, Deubiquitinase, DNA repair, Histones

Background information: Deubiquitinases (DUBs) are a poorly understood family of enzymes that catalyze the reverse reaction of ubiquitination. These proteins control cell cycle, gene expression, DNA damage response (DDR) and other cellular processes, deregulation of which play crucial roles in leukemia development. Accordingly, targeting DUBs might prove to be a promising approach for developing novel anti-cancer therapies.

Recent studies identified USP16 deubiquitinase as a regulator of Hox gene expression through H2A deubiquitination. Consistent with an important role in cell function, USP16 gene mutations encoding abnormal protein fusions has been identified in Chronic Myelomonocytic Leukemia (CML). Previous studies indicated that USP16 is predominantly cytoplasmic, but exerts nuclear functions. Our study tried to determine the mechanism that coordinates USP16 nucleo-cytoplasmic transport and its implication in Leukemia apparition.

Methods: In these studies, we used molecular and cellular biology approaches applied to normal or tumoral cells. We employed CRISPR-Cas9 knock out system (KO) to inhibit USP16 and analyze the molecular and cellular consequences. We also mutated various important domains of USP16 and determine the impact of these mutations on its localization, H2A deubiquitination and DNA damage response.

Results: Our studies showed that USP16 is mainly cytoplasmic and contains an atypical nuclear localization signal (NLS) suggesting that this DUB is highly regulated at the level of nucleo-cytoplasmic transport. Interestingly, we observed protracted accumulation of USP16 in the nucleus when the cells are treated with leptomycin B, an inhibitor of nuclear export. This nuclear translocation of USP16 is correlated with a drastic reduction of H2A ubiquitination levels. We also defined a mechanism whereby a strong nuclear export signal (NES) is responsible for the cytoplasmic retention of USP16. Our analyses also revealed that USP16 lacking the NES strongly abolishes the double strand DNA repair pathway through downregulation of DNA damage signaling and the ubiquitin ligase RNF168. In addition, in response to ionizing radiations, USP16 KO cells showed increased gH2AX indicating a delay in DNA repair.

Conclusion: Our results provide insights into the complex nature of USP16 subcellular localization and its role in modulating H2A ubiquitination and DNA damage response. This study significantly contributes to the understanding of how the ubiquitin system coordinates DNA repair pathways. As the USP16 gene is translocated in leukemia, our study might help identifying novel inhibitors for the treatment of this disease.

P07

Title: Mysm1 as a potential drug target for p53 activation in B cell malignancies.

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Keywords: Hematopoiesis, p53, B cell, H2A deubiquitinase, lymphoma

Background: MYSM1 is a chromatin binding histone deubiquitinase and MYSM1 mutations in humans result in lymphopenia, while loss of MYSM1 in mice causes severe hematopoietic abnormalities, including an early arrest in B cell development. MYSM1 deficiency is accompanied by increased p53 levels and an activation of p53-mediated stress response in the bone marrow, whereas knock out of the p53 gene completely rescues the defect in hematopoiesis observed in MYSM1 knock out animals.

Purpose: With this study we aimed to analyze a) the role of MYSM1 at different checkpoints in B cell development and B cell mediated immune responses b) the role of MYSM1 as a potential drug target for p53 activation in B cell lymphoma

Results: We analyzed conditional mouse models inactivating Mysm1 at pre-pro-B, pre-B, and follicular B cell stages of development. We show that loss of MYSM1 at the pre-pro-B cell stage results in impaired B cell differentiation, with reduction in B cell numbers in lymphoid organs. In these mice B cells also showed increased activation and class switching, together with impaired survival and proliferation. In contrast, MYSM1 was largely dispensable from the pre-B cell stage onwards.

When we crossed MYSM1 deficient mice, with IgH-Myc transgenic animals that spontaneously develop pre pro B cell lymphomas, we saw improved survival of Mysm1-/-Tg.IgH-Myc animals compared to their respective littermate controls.

Conclusion: Our work identifies Mysm1 as an essential regulator of B cell lineage specification underscoring the importance of MYSM1 activity at the pre-pro-B cell stage for normal responses to antigen stimulation once they complete their maturation process.

In addition, our preliminary data concerning Mysm1-/-Tg.IgH-Myc indeed points to Mysm1 as a new therapeutic target for B cell malignancies.

P08

Title: Mnk1 promotes in the progression of breast ductal carcinoma in situ to invasive ductal carcinoma

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Keywords: cancer invasion, mRNA translation, MNK1, eIF4E

Background information: Ductal carcinoma in situ (DCIS) is referred to as "stage 0" disease. The standard care of DCIS includes surgery, radiation and sometimes hormone therapy. In some cases, DCIS progresses to become invasive ductal carcinoma (IDC). Thus, DCIS is over-treated because some patients never progress to IDC. It is important to understand the underlying mechanism for the DCIS to IDC transition, in order to better treat patients. DCIS and IDC share similar genomes and transcriptomes, thus we hypothesize that aberrations in mRNA translation contributes to DCIS-IDC conversion.

Purpose of the study: We propose to study whether aberrant activation of the Mnk/eIF4E axis, known to regulate pro-tumor/pro-invasive mRNAs, promotes IDC.

Methods: We have engineered DCIS cells (MCFDCIS.com) to express control vector pBABE (DCIS-pBABE) or constitutively active MNK1 (DCIS-caMNK1). *In vitro* experiments including 3 dimensional culture, migration and invasion assays, colony formation assay and propidium iodide staining/cell cycle analysis are performed to characterize the potential outcome of caMNK1 overexpression and the effects of MNK inhibitor SEL201. To determine whether caMNK1 facilitates tumor outgrowth and DCIS to IDC transition, *in vivo* experiments are performed by mammary fat pad xenografting of DCIS-pBABE or caMNK1 into SCID or nude mice. H&E staining and IHC staining of MNK1, phosho-MNK1, p63 are performed to determine the morphology of tumor xenografts.

Results: DCIS-caMNK1 cells gain increased tumorigenic and invasive properties, such as: (1) increased colony formation, (2) larger acini size in 3D culture, and (3) increased migration and invasion. Moreover, a novel Mnk1/2 inhibitor, can inhibit proliferation, colony formation, 3D acini formation, and migration and invasion of DCIS-pBABE and -caMNK1 expressing cells. Importantly, DCIS-caMNK1 tumors grew faster comparing to DCIS-pBABE controls. Additionally, nude mouse experiments revealed that DCIS-caMNK1 expressing tumors progress to IDC, while DCIS-pBABE derived tumors retain DCIS-like structures.

Conclusion/Future work: MNK1 may play an important role in promoting DCIS to IDC transition. To further validate our *in vivo* and *in vitro* results, samples from patients with DCIS, IDC or mixed DCIS-IDC will be obtained to examine the levels of phospho-Mnk1 and phospho-eIF4E.

P09

Title: miR-17~92 is a global metabolic regulator in Myc-driven lymphoma

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Keywords: Myc, miR-17~92, Metabolism, Lymphoma, LKB1

Background: Myc is a protooncogene widely implicated in cancer and acts to engage an array of biological functions. Among the many biological processes sensitive to Myc activity is cellular metabolism. Given Myc's prolific transcriptional activity, the targets genes responsible for the promotion of anabolic metabolism are only marginally understood. Downstream of Myc is the miR-17~92 microRNA (miRNA) cluster, a polycistronic gene that encodes six mature miRNAs. miR-17~92 was found to cooperate with Myc to promote more aggressive lymphoma development in an Eμ-Myc model of Burkitt's lymphoma. Conversely, deletion of miR-17~92 severely diminished the tumourigenic potential of Eμ-Myc lymphoma cells, suggesting that miR-17~92 acts as a key mediator of the Myc oncogenic program.

Purpose: Given the powerful regulatory effects of Myc on cellular metabolism, and the important role miR-17~92 plays in mediating the tumourigenic potential of Myc-driven lymphoma, we sought to determine whether the pro-oncogenic effects of miR-17~92 is driven, in part, by the ability to regulate cellular metabolism in lymphoma.

Methods: Eμ-Myc lymphoma cells harboring conditional deletion alleles of miR-17~92 were employed. Following deletion of miR-17~92, metabolic parameters were measured using the Nova bioanalyzer for media metabolite concentrations, and the Seahorse extracellular flux analyzer that serves to measure extracellular acidification and oxygen consumption rates. Metabolomics were also employed to gauge intracellular metabolite dynamics using gas chromatography coupled to mass spectroscopy.

Results: To date, we have shown that miR-17~92 is an essential mediator of the metabolic reprogramming engendered by Myc in lymphoma. Deletion of miR-17~92 in Eμ-Myc lymphoma cells led to large reductions in glycolytic and glutaminolytic metabolism, which are hallmarks of elevated Myc expression in cancer. RNA-seq analysis of the wild type parental cells (fl/fl) or cells lacking miR-17~92 (Δ/Δ) found large reductions in genes categorized as being components of oxidative metabolism, the TCA cycle, and glycolytic metabolism. Of the six mature miRNAs produced by the cluster, the miR-17 family (miR-17/20) was found to contribute the most to promoting glycolytic and glutaminolytic metabolism, and cells lacking miR-17/20 (Δ/Δ +Δ17,20) were not able to fully rescue the defects in tumourigenicity displayed by the Δ/Δ cells. We have shown that the tumour suppressor and master metabolic regulator LKB1 is a target of the miR-17 family, and this repression is important in the control of metabolism in Myc-driven lymphoma.

Conclusion: We have found that the powerful metabolic reprogrammer and oncogene Myc depends on the miR-17~92 cluster for this regulatory activity. Given that miRNAs act as regulators of gene expression at the translational level, we found the novel mir-17 target LKB1 largely explains the metabolic regulatory activity of miR-17~92. Because LKB1 acts to restrain anabolic metabolism in cancer, a reduction in LKB1 expression would be predicted to sensitize highly metabolically active cancer cells to metabolic stressors. To this end, we have begun testing novel small molecule metabolic stressors to gauge their efficacy in treating lymphoma.

P10

Title: Molecular mechanisms of spatio-temporal regulation of the Polo kinase in cell division

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Keywords: Cell cycle, Mitosis, Polo kinase, Leukemia, Drosophila

Background information: Cancer is characterized by excessive cellular proliferation. The Polo kinase is an essential regulator of mitosis and cytokinesis and its inhibition causes mitotic catastrophe and cell death. The Polo kinase (called Plk1 in Humans) is overexpressed in a variety of malignancies including pediatric acute myeloid leukemia (AML) and serves as a negative prognostic marker in specific human cancer types.

Purpose of the study: Polo is composed of an N-terminal Ser/Thr kinase domain (KD) and a C-terminal Polo-Box domain (PBD), which mediates protein interactions with substrates and localization to different subcellular structures. In addition, the two domains of Polo inhibit each other by an intramolecular interaction. The multiple functions of Polo are enabled by a complex regulation of its activities that is not completely understood The purpose of this study consists to understand how the spatio-temporal regulation of the Polo kinase controls its cellular functions?

Methods: We have investigated this question using *Drosophila melanogaster* as a model and its cells in culture. We used both genetics, biochemistry (coIP, kinase assays) and cellular biology (immunofluorescence, live cell imaging) approaches to decipher the regulation of the Polo kinase during cell division.

Results: To dissect Polo functions and regulation *in vivo*, we generated transgenic flies expressing different forms of Polo fused to GFP. The first 13 nuclear divisions in Drosophila syncytial embryos are rapid and synchronous and allow clear and detailed live imaging of the events of mitosis. In this tissue, we have examined the effects of different mutations in Polo on its subcellular localization through mitosis. We found that phosphorylation of Polo at its activation loop is required for its nuclear translocation in prophase. We are dissecting the molecular mechanism underlying this requirement in flies and cell culture. Genetic rescue experiments of Polo RNAi in different tissues of the fly are informing on the functional importance of the regulatory mechanisms identified.

Conclusion: Collectively, our results illuminate the molecular mechanisms that regulate the Polo kinase during cell division and may suggest new therapeutic avenues that will disrupt Polo functions in cancer cells.

P11

Title: Chemo-genomic interrogation of primary acute myeloid leukemia with biallelic *CEBPA* mutations reveals recurrent *CSF3R* mutations and subgroup sensitivity to JAK inhibitors

Author: Vincent-Philippe Lavallée, Clinician 2014-2016

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Keywords: AML, CSF3R, CEBPA, chemical screen, transcriptomics

Background information: Acute myeloid leukemias (AML) with *CEBPA* mutations define a provisional entity in the WHO 2008 classification. Patients with biallelic *CEBPA* (*CEBPA*^{bi}) mutations comprising N-terminal frameshift and C-terminal in frame mutations (hereafter termed typical *CEBPA*^{bi} AML) characteristically present a normal karyotype and have a favorable clinical outcome. Frequency and nature of co-occurring mutations are still largely unknown in this subgroup for which no targeted therapy is available.

Purpose of the study: Using the RNA-sequencing data from 415 primary AML in Leucegene cohort, we aimed to refine the transcriptomic and mutational landscape of 14 *CEBPA*^{bi} AML samples present in this collection, and to interrogate the novel mutations identified in this subgroup in a targeted chemical inhibitor screen.

Methods: We performed RNA-sequencing, comparative transcriptomic analysis, mutation detection, cell culture and chemical screening using the methods previously reported (Lavallée VP *et al*, Nature Genetics, 2015).

Results: In our cohort of *CEBPA*^{bi} AML we identified 23 mutated genes, including the previously reported mutations in *WT1* and *GATA2* in 3/14 (21% each). The most frequent mutations in *CEBPA*^{bi} AML was the activating *CSF3R* T618I mutation present in 29% (4/14) of this subgroup compared to only 3/401 *CSF3R* mutated samples in other AML subtypes ($p < 0.0001$). *CSF3R* encodes the granulocyte colony stimulating factor receptor (G-CSFR) and is a direct target of CEBPA, suggesting a selective pressure for acquisition of these mutations in *CEBPA*^{bi} AML cells. We next conducted a targeted chemical screen employing a collection of compounds (n=11) enriched for JAK inhibitors. Inhibitors were tested in a dose response assays using *CEBPA*^{bi} (n=14) and control normal karyotype *CEBPA* wild-type (NK *CEBPA*^{wt}, n=14) primary AML cells. Results showed that all *CSF3R* T618I mutated samples were sensitive to JAK inhibitors (e.g. median ruxolitinib IC_{50} = 66nM (range: 48 - 94)). Most interestingly, all *CEBPA*^{bi} samples with a characteristic gene expression profile (n=11), irrespective of their *CSF3R* mutation status, were uniformly and significantly more sensitive than NK *CEBPA*^{wt} specimens to JAK inhibitors (e.g. ruxolitinib median IC_{50}: 62 vs 181 nM, $p = 0.01$), but not to other inhibitors.

Conclusion: Our study reports a novel co-occurrence of mutations within the *CEBPA*/*CSF3R* pathway in *CEBPA*^{bi} AML and reveals a uniform sensitivity to JAK inhibitors in *CEBPA*^{bi} AML cells. This may suggest that networks upstream of JAK-STAT are aberrantly activated in a majority of these specimens. Altogether, it paves the way to personalized clinical trials repositioning JAK inhibitors for *CEBPA*^{bi} AML.

P12 ***(Absent)***

Titre: Pratique d'activité physique et aptitude aérobie de survivants de leucémie lymphoblastique aiguë

Auteurs: Valérie Lemay, PhD 2014-2016

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Mots clés: physiologie de l'exercice, cardiotoxicité, leucémie lymphoblastique aigüe, activité physique

Introduction: Le taux de survie des enfants atteints de leucémie lymphoblastique aigüe (LLA) se chiffre maintenant à environ 85%. Malheureusement, plus de 2/3 des survivants sont confrontés plusieurs années à la fin de leurs traitements à des problèmes de santé chroniques (obésité, troubles métaboliques, densité osseuse réduite, problèmes cardiaques). Plusieurs études suggèrent aussi que l›aptitude aérobie (VO_2peak) des survivants LLA est réduite par rapport à la population générale. Comme une bonne aptitude aérobie est un prédicteur de mortalité toute cause, il est important de mieux comprendre la provenance de cette réduction de VO_2peak chez les survivants LLA.

Buts de l'étude: 1) Présenter un portrait détaillé de la pratique d'AP des survivants de LLA et établir des liens avec leur VO_2peak et les traitements reçus; 2) Présenter la réponse à l'effort maximal des survivants LLA; 3) Vérifier la participation de l'aptitude physique dans la prévention des comorbidités post-traitements.

Méthode: 250 survivants LLA (51% hommes, 22 ± 6 ans, 57% risque LLA élevé; 43% risque LLA standard) ont été évalués au CHU Sainte-Justine 14 ± 5 ans après la fin de leurs traitements. Ils ont rempli un questionnaire de rappel d›activités physiques et sédentaires et ont complété une épreuve d›effort maximale sur bicyclette ergométrique. Les paramètres cardiorespiratoires à l›effort ont été mesurés à l›aide d›un analyseur de gaz et les paramètres hémodynamiques ont été mesurés avec un ECG 12 dérivations, prises de tension artérielle et cardiographie d›impédance.

Résultats: Les données préliminaires recueillies auprès des 190 premiers participants indiquent qu'ils pratiquent en moyenne 30 ± 32 minutes d'AP de loisirs par jour et consacrent 226 ± 160 minutes quotidiennement aux activités sédentaires (télévision, ordinateur, jeux vidéos et lecture). 69% ne respectent pas les recommandations canadiennes en matière de pratique d'AP. Seulement la catégorie de poids s'est révélée être un prédicteur de la pratique d›AP (min/jour). Le pourcentage de déconditionnement (valeur mesurée de VO_2peak vs valeur prédite) moyen est de -14 ± 16%, avec plus de 45% des participants présentant un déconditionnement égal ou supérieur à -15%. Même chez les sujets respectant les recommandations d›AP, le pourcentage de déconditionnement est de -7±17%. Les sujets LLA à risque élevé ont une VO_2peak significativement inférieure aux sujets à risque standard.

Conclusion: Les résultats des analyses préliminaires appuient les études existantes suggérant un déconditionnement chez les sujets LLA comparativement à la population normale. Ce déconditionnement s›explique d›une part, par une pratique insuffisante d›AP, mais aussi possiblement par des facteurs physiologiques relatifs aux traitements reçus. D›autres analyses plus détaillées, notamment au niveau des paramètres ventilatoires et hémodynamiques à l›effort, permettront de mieux comprendre les possibles limitations physiologiques retrouvées chez cette population. L›impact préventif d›une bonne aptitude aérobie sur les comorbidités observées à long terme sera également évalué. Les résultats obtenus jusqu›à maintenant soulignent l›importance de développer des programmes d›intervention pour promouvoir la pratique d›activité physique de ces survivants LLA.

P13

Title: PRMT5 regulates B cell development and germinal center homeostasis

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Keywords: Arginine Methylation, B cells, PRMT5, germinal center, B cell lymphoma

Aim of the study: B cells develop in the bone marrow, where immunoglobulin genes are rearranged leading to the expression of a functional B cell receptor. Mature B cells then populate the periphery, where their activation by cognate antigen prompts the formation of germinal centers (GC). GC B cells undergo somatic hypermutation, underpinning affinity maturation, and isotype switching. Arginine methylation is a post-translational modification that affects many cellular functions but is understudied in B cells.

Purpose of the study: We want to investigate the role of protein arginine methyl transferase 5 (PRMT5) in B cells.

Methodology: We analyzed mice in which PRMT5 is ablated during B cell development in the bone marrow or in activated mature B cells.

Results: PRMT5 ablation in pro-B cells causes a total block in development at that stage. In immunized mice, removal of PRMT5 in activated B cells reduces formation of GC B cells three-fold, and completely blocks antigen-specific antibody production. *Ex vivo*, depletion of PRMT5 in activated mouse primary B cells reduces class switch recombination, cell proliferation and viability.

Conclusions: We find that PRMT5 is necessary for B cell development and GC B cell homeostasis. We are now investigating the mechanism of action behind these phenotypes. Interestingly, PRMT5 is overexpressed in B cell lymphomas, so our research will lead to a better understanding of PRMT5's role in both normal and malignant B cells.

P14

Title : Structure/Function Analysis of the c-MYC Proto-oncogene mRNA

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Keywords : MYC, 5'UTR, CRISPR/Cas9, genome editing, translation

Background: In the past decades, several elements of the cap-dependent translation apparatus have been associated with tumorigenesis and/or chemoresistance. Briefly, during cap-dependent initiation, the eukaryotic initiation factor 4F (eIF4F) complex is recruited to the 5' cap of mRNAs. This step is rate-limiting for translation and a check point for linking PI3K/mTOR and Ras-MAPK signalling activity to protein synthesis. Structural barriers in mRNA 5' untranslated regions (5'UTR) increase their dependency on eIF4F activity. Among transcripts with long, structured 5' UTRs is MYC, an extensively studied transcription factor, known to be an eIF4F-responsive mRNA. Translocations, some of which truncate the MYC 5' UTR, lead to its overexpression and are known drivers in cancers such as Burkitt's lymphoma and childhood medulloblastoma.

Purpose of the study: The aim of this study is to identify elements in the MYC 5' UTR that may serve as barriers to its translation.

Methods: In the pursuit of this objective, we performed an *in vivo* screen using the CRISPR/Cas9 system targeting MYC 5'UTR. Previous experiments performed in the *Eu-Myc* mouse model have shown that p53 suppression cooperates with elevated MYC levels to induce lymphomagenesis. We thus co-transduced C57BL/6 hematopoietic stem cells (HSC) with guide RNAs targeting the MYC 5'UTR (sgMyc) as well as a small hairpin RNA suppressing p53 expression (shp53). These HSCs were then implanted into wild-type C57BL/6 mice. Should targeting of the 5'UTR of MYC lead to increased expression, we should be able to observe cooperativity with depletion of p53 and acceleration of tumorigenesis.

Results: In the course of this screen, several recipients developed tumors. Sequencing of genomic DNA revealed important CRISPR-Cas9 induced genome editing at the target site in three tumors generated from two different sgMYC pairs. These tumors contained high levels of Myc protein and mRNA. However, investigation of the 5'UTR sequence of MYC mRNA in those tumors revealed a retroviral integration at Cas9 cut sites. The sequence integrated in the 5'UTR of MYC in those tumors came from the retroviral vector used to introduce shp53 into HSCs. The hairpin expression in that vector was driven by the virus long terminal repeat (LTR). Integration of this sequence in MYC 5'UTR drove the expression of the oncogene, leading to its overexpression and thus tumorigenesis.

Conclusion: We have developed a screening method to target mRNA 5'untranslated region. Although Myc overexpression was observed in those tumors, it originated from viral integration in MYC 5'UTR. These results highlight a drawback of retroviral screens using CRISPR/Cas9 in that the genome editing activities of Cas9 may provide recombinogenic sites for retroviral integration. The use of self-inactivating (SIN) vectors in future screens should minimize such potential undesired gene activation events.

P15

Title: Characterization of RET in MLL-AF9 acute myeloid leukemia

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Keywords: Leukemia, Genomics, Molecular Biology,

Background information: Translocations involving the *MLL* gene (11q23) are among the most frequent lesions in pediatric hematologic malignancies. Moreover, they confer a poor prognosis and exhibit the unique ability to generate both AML and ALL leukemias. In collaboration with the lab of Dr. Frederic Barabé (Université Laval), we have generated a novel human leukemia model using the MLL-AF9 (MA9) fusion gene, a common partner gene in MLL translocations. This model uses lentiviral transduction of the MA9 oncogene into healthy single donor cord blood (CB) stem cells which are xenografted into immuncompromised (NSG) mice, allowing the generation of both AML and ALL leukemias. Through stepwise RNA-seq, we have found 39 differentially expressed genes in MA9 AML leukemias (both patients and models) in comparison to untransduced CB cells. One of these genes, RET, is a receptor tyrosine kinase (RTK) known for its oncogenic role in endocrine cancers such as medullary thyroid carcinoma, with no previously reported functional role in AML.

Purpose of the study: Considering RET's oncogenic role in other cancers, we aim to determine its importance in AML. Expression studies show that its known signaling partners are not expressed in AML, suggesting we also need to investigate its downstream partners for novel signaling mechanisms.

Methods: In order to validate the role of RET as an oncogene, we are taking advantage of shRNA-mediated knockdown in AML cell lines to determine the importance of RET in leukemogenesis . Furthermore, we are investigating the RET downstream signaling by using a previously described EGFR-RET chimeric receptor. The addition of EGF in the culture media allows activation of RET signaling in a more controlled setting and study the effects downstream. By mutating the cytoplasmic tail of either the endogenous or the chimeric receptor, we can determine which portions and residues are in fact essential for the leukemic effect. We are characterizing the impact of these mutations by luciferase reporter assay and Western Blot on known RET targets, such as β-catenin, ERK1/2 and AKT. Potential new partners will also be investigated following co-immunoprecipitation of RET by using mass spectrometry.

Results: Lentiviral transduction of RET specific shRNA in MA9 AML cell lines THP-1, NOMO-1 and MOLM-13 causes significant reduction in their viability, supporting the oncogenic role of RET. In contrast, RET knockdown in non MA9 AML line KG1a has no impact on viability, suggesting a MA9-specific phenotype. Moreover, shRNA knockdowns of RET in MA9 AML cells also improves NSG mouse survival following cell line transplantation, further supporting our hypothesis.

Conclusion: The oncogene RET, which is overexpressed in MA9 AML, seems to be a potent regulator of leukemogenesis. Thus, this project will not only understand which portions of this receptor are more suited for targeted inhibition, but what are the signaling partners involved in leukemogenesis. This approach is key to the development of any novel potential RET inhibitors for the treatment of AML.

P16

Title: Expression of immunoproteasome genes is regulated by cell-intrinsic and –extrinsic factors in human cancers

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Key words: Proteasome, Immunoproteasome, Protein stress, Acute Myeloid Leukemia

Background Information: Consistent with the fact that cancer cells endure proteotoxic stress, proteasome inhibitors are used in clinical to treat multiple myeloma. In addition to constitutive proteasomes (CP), vertebrates express immunoproteasomes (IP), which display distinct substrate preferences.

Purpose of the study: IP are not ubiquitously expressed as compared to CP. Furthermore, cells with IP deletion or mutations show that IP is implicated in managing cells stress by removing damaged proteins.

Methods: Transcriptomic analysis of thousands of cancer samples from The Cancer Genome Atlas were performed along with correlation with clinical data.

Results: While expression of CP genes did not correlate with prognosis, high expression of IP genes was associated with longer survival in breast cancer and shorter survival in acute myeloid leukemia (AML). In breast cancer, expression of IP genes was determined by the abundance of tumor infiltrating lymphocytes. In contrast, expression of IP genes in AML was a cell-autonomous and IFN-independent feature and was particularly high in AML with an M5 phenotype and/or *MLL* rearrangement. Notably, PSMB8 inhibition led to accumulation of polyubiquitinated proteins and cell death in IP^{high} but not IP^{low} AML cells. Co-clustering analysis revealed that in M5 AMLs were primarily co-regulated with genes involved in cell metabolism and proliferation, mitochondrial activity and stress responses.

Conclusion: We conclude that M5 AML can upregulate IP genes in a cell-intrinsic manner in order to resist cell stress. Furthermore, based on the increased reliance of AML cells on oxidative phosphorylation, we speculate that IP upregulation in AML cells is driven by oxidative stress.

P17

Title: *Ezh1* and *Ezh2* are essential regulators of normal and leukemic hematopoietic stem cells

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Keywords: EZH2, PRC2, Leukemia, Epigenetic, Hematopoiesis

Background: A growing number of evidence predicts similarities in the genes that regulate self-renewal of normal and cancer stem cells. Promising candidates include the Polycomb group (PcG) family of genes, which play a role in both stem cell self-renewal and in cancer. The canonical polycomb PRC2 complex, which displays methyltransferase activity on lysine 27 of histone H3, contains two alternative catalytic subunits: EZH1 or Ezh2. It has become evident that in various blood cancers, the PRC2 genes are frequently mutated, suggesting that defective PRC2 activity can be fundamental in the aetiology of haematological malignancies. Conversely, other blood cancers such as AML and B-ALL require an intact PRC2 complex to maintain their leukemogenecity.

Purpose of the study: The aim of this study is to understand the function of the catalytic subunits of the PRC2 complex, namely Ezh1 and Ezh2, in normal hemopoietic and leukemia stem cells. We put a focus on the identification of redundant functions between these two subunits.

Methods: We recently generated an *Ezh2/Ezh1* (Ezh1/2) conditional double KO (DKO) mouse model in order to completely abrogate the catalytic activity of the PRC2 complex in the hematopoietic cells. We used competitive transplantation assays to evaluate the self-renewal and the proliferation potential of mutant hematopoietic stem cells. We also performed rescue-type experiments with mutant *Ezh2* constructs in order to determine which domains are essential to its function. Finally, we retrovirally transduced hematopoietic cells from transgenic animals with *Hoxa9 and Meis1* oncogenes evaluated theirs ability to induce myeloid leukemia in recipient animals.

Results: While *Ezh1* or *Ezh2* inactivation alone does not induce a dramatic effect (*Ezh2* inactivation is mainly affecting B and T cell differentiation while *Ezh1* inactivation is slightly detrimental for hematopoietic stem cells self renewal), concomitant inactivation of *Ezh1/2* in the blood system leads to aplastic anemia and bonne marrow failure within two weeks. Competitive assays have shown that hematopoietic stem cell originating from double KO mice are not able to compete with their wild type counterparts and will rapidly disappeared from the recipient animal. Re-introduction of wild type Ezh2 but not a catalytic dead mutant is able to completely restore cell survival. Finally, *Ezh1/2* co-inactivation is able to greatly delay the *HoxA9 Meis1* leukemia onset in comparison with *Ezh2* inactivation alone, while *Ezh1* inactivation do not give any advantages compared to WT *HoxA9 Meis1* leukemia.

Conclusion: Together, these studies show that Ezh1/2 are essential regulator of normal and leukemic hematopoietic stem cells, likely through their methyltransferase activity. We also showed that EZH1 and EZH2 share redundant functions essential for bone marrow homeostasis and cell survival.

P18

Title: Chemogenomic approach to characterize acute myeloid leukemia with complex karyotype

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Keywords: Leukemia; complex karyotype; prognostic tool; marker of poor prognosis; chemogenomics

Background information: Acute myeloid leukemia (AML) patients with complex karyotype (≥3 chromosomal abnormalities) represent 20% of AML and are generally associated with poor outcome. It is a heterogeneous subgroup with patients carrying various mutations and chromosomal abnormalities. *TP53*, considered as the "guardian of the genome", is mutated in about 50% of these patients. Better characterization of this subgroup is needed to improve the diagnosis and therapeutic orientation of these patients.

Purpose of the study: As part of the LEUCEGENE project initiated at IRIC we analyzed 68 complex karyotype AML primary samples on different aspects (mutational status, cytogenetics, gene expression profiles, sensitivity to chemicals *in vitro*) in order to refine the complex karyotype AML classification and improve the diagnostic tools.

Methods: Genetic abnormalities and transcriptome data were determined by next generation sequencing for 68 AML primary samples with complex karyotype (from BCLQ: Banque de Cellules Leucémiques du Québec) including 46 altered-TP53 samples and correlated with clinical data. Mutations were validated by Sanger sequencing. Transcriptome data of these patients were compared to 347 non-complex AML from the BCLQ. A chemical screen was run on 38 samples cultured in optimized AML medium with 270 selected drugs. Samples were selected according to their TP53 status and gene H expression level.

Results: AML patients with complex karyotype form a heterogeneous subgroup with various genetic abnormalities. The most recurrent mutated gene is *TP53* in about 50% of the patients, followed by *RUNX1* mutation. No new recurrent mutation was identified. Looking at the transcriptional signature of these patients we identified *gene H* as a marker for complex karyotype AML and more generally as a marker of poor prognosis in AML. About 30% of the patients from our whole cohort of AML (n=415) express a high level of *gene H*. This subgroup is highly enriched in complex karyotype and altered-*TP53* AML and shows a drastic reduction of overall survival rate.

Within the complex karyotype AML cohort, various *gene H* expression levels and *TP53* status of the patients allow the classification of the patients into distinct subgroups with different transcriptomic signatures and different response to treatment. Complex karyotype AML patients expressing *gene H* and carrying a functional *TP53* are interestingly highly sensitive to the family of drugs X that all target the same pathway compared to the rest of the samples.

Conclusion: We describe a large cohort of complex karyotype AML patients. We identified *gene H* as a marker of poor prognosis in a subgroup of patients highly enriched in complex karyotype. The detection of this marker in AML would allow identifying 30% of patients that do not respond to standard treatments and who could benefit from investigational approaches. Interestingly complex karyotype AML samples expressing *gene H* with a functional *TP53* are sensitive to a group of drugs targeting different members of the same pathway. The analysis of a large group of complex karyotype AML allows the characterization of distinct subgroups of patients that show different transcriptomic signature and different response to treatment. This study gives us a better understanding of the complex karyotype AML and would lead to a more appropriate therapeutic care of these patients.

P19

Title: A chemo-genomics approach to dissect and increase self-renewal divisions of hematopietic stem cells

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Keywords: Graft optimization, hematopoietic stem cells, ex-vivo expansion, UM171.

Background information: Hematopoietic stem and progenitor cell (HSPC) transplantation constitutes one of the most effective therapeutic strategies for patients with leukemia and bone marrow failure. HSC are able to restore a healthy bone marrow through their ability to sustain lifelong production of all mature blood cells in vivo. Human cord blood (CB) transplantation is ideal to this purpose, thanks to its immature immune system and rapid availability. However, as most CB grafts suffer from low cell numbers insufficient for adults, expansion of HSPC becomes of great interest. The small molecule UM171, recently identified in the laboratory, is able to expand primitive cells in culture and represent, therefore, an important tool to understand the molecular basis for HSC self-renewal and translate this know how to bone marrow transplantation clinics.

Purpose of the study: The aim of this research project is to use chemogenomic and proteomic approaches to uncover cellular pathways which are essential to HSC proliferation and to identify new small molecules synergizing with UM171 in increasing HSPC homing and expansion.

Methods and Results: Signaling pathways rapidly activated by UM171 and connected to the expansion of the primitive CD34+ CD45RA- population have been evaluated by means of a phospho-proteomic approach by stable isotope labeling with amino acids in culture (SILAC). Lysates from cells treated with UM171 or the vehicle at specific time points have been proteolytically digested and analyzed by LC-MS/MS. Results obtained in both CD34+ CB cells and the AML5 cell line (which is responsive to UM171) have been compared, revealing a rapid induction of the mTOR pathway by UM171 treatment. The involvement of this pathway in UM171-mediated effect in HSPCs biology is currently under analysis.

In an attempt to identify additional small molecules that could potentiate the effects of UM171 on HSC expansion, we carried out a HTS-FACS-based *in vitro* screen on expanded CB in the presence of UM171. Two small molecules, UM092 and UM827, were identified based on their ability to increase the proportion of CD34+ cells. Preliminary results revealed that UM092 and UM827, respectively through the inhibition of the aryl hydrocarbon receptor and to the increase of the membranal cKit receptor, collaborates with UM171 to enhance the percentage and absolute numbers of $CD34^+$ and more primitive $CD34^+CD45RA^-$ CD90+ cells recovered after culture.

Conclusions: Overall, our results allowed a better comprehension of UM171's ability to expand hematopoietic stem cells and to the definition of better culture conditions for HSPC in vitro culture. Our data not only will expand our knowledge about the biology of hematopoietic stem cells but might also lead to the production of optimized cord blood grafts which will be readily translated to the clinic.

P20

Title: Interleukin-21 accelerates T-cell reconstitution following allogeneic bone marrow transplantation or human cord blood transplantation.

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Keywords: Interleukin-21; Bone marrow transplantation; GVHD; Immune reconstitution.

Background information: Allogenic bone marrow (BM) transplantation (BMT) is currently used as a treatment for hematological malignancies. However, associated pre-conditioning regimens trigger substantial thymic damages. As a consequence, the thymus is no longer able to sustain the generation of naive T cells, thus keeping the host in a complete immunodeficiency state ultimately leading to further fatal ailments. Such unmet medical need demonstrates the central importance in identifying new factors able to stimulate intrathymic T-cell development. Unexpectedly, IL-21 was recently found to display thymopoiesis-stimulating activity.

Purpose of the study: Accordingly, we hypothesized that its use can accelerate T-cell reconstitution following allogenic BMT.

Methods: As a model, we transplanted irradiated LP/J mice with T-cell-depleted BM from RAG2p-GFP mice. The use of RAG2p-GFP mice model (C57BL/6 background) allows us to follow thymopoïesis by analyzing the GFP level, which is reflective of newly developed T cells. In order to study GVHD, mice were transplanted as previously but purified splenic-T cells were added to BM cells. To complete our study, we used another mouse model related to human: the NOD scid gamma (NSG) mouse model. We transplanted these immunodeficient mice with human hematopoietic stem cells purified from human cord blood. The use of these human hematopoietic stem cells allows the generation of a human immunological system in mice.

Results: A weekly analysis of GFP+ T cells in peripheral blood demonstrates that IL-21 accelerates T-cell generation two weeks earlier than control mice. Furthermore, thymic analysis of IL-21-treated mice shows similar absolute number of total or GFP-expressing thymocytes compared to untransplanted RAG2p-GFP mice. Although newly generated T cells are functional, no signs of graft-versus-host disease were triggered in IL-21-treated mice transplanted with BM supplemented with C57BL/6-derived allogeneic T cells. This protective effect is believed to be caused by the IL-21-induced generation of regulatory B cells. A similar reconstitution kinetic was observed in transplanted NSG mice receiving human IL-21 clearly showing a potent effect for IL-21 in de novo thymopoiesis.

Conclusion: In sum, our results epitomize IL-21 as a new pharmacological compound able to trigger rapid recovery of thymic functions in subjects undergoing allogeneic BM or stem cell transplantation.

P21

Title: Pre-TCR/CD3 signalling at the **β**-selection checkpoint determines disease onset and penetrance in T acute lymphoblastic leukemia (T-ALL) induced by SCL-LMO1 oncogenes

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Keywords: pre-TCR/CD3 complex signalling, β-selection checkpoint, E proteins, T-acute lymphoblastic leukemia

Background information: Pre-TCR/CD3 signalling, together with high Notch1 activity, is essential for thymocyte survival and differentiation at the β-selection checkpoint, when it triggers clonal expansion accompanied by a burst of cell proliferation in the thymus. The signal that emanates from the pre-TCR/CD3 complex drives T cell proliferation by inhibiting the activity of E proteins transcription factors (E2A and HEB), which act as gatekeepers at the β-selection checkpoint and have tumor suppressor functions in T acute lymphoblastic leukemia (T-ALL). While the molecular events at the β-selection checkpoint are required for normal thymocyte development, their role in leukemogenesis remains to be addressed.

Purpose of the study: To characterize the role of the pre-TCR/CD3 complex signalling and downstream inhibition of E proteins as a driving oncogenic pathway in T acute lymphoblastic leukemia (T-ALL).

Methods: We combined the genetics of the mouse with functional genomics to address this question. Specifically, we investigated T-ALL development in mouse models with both functional (*Cd3*e$^{+/+}$) and deficient pre-TCR signalling (*Cd3*e$^{-/-}$) expressing the human *SCL* and *LMO1* oncogenes. In order to gain insights into the molecular events required to T-ALL progression, we performed transcriptome profiling of normal thymocytes, pre-leukemic and fully transformed T-ALL obtained from mouse models.

Results: We show that the penetrance of SCL-LMO1 induced T-ALL decreased from 100% (wild-type mice) to 36% in pre-TCR deficient mice, and the disease onset was significantly delayed by ~70 days. Overall, *Cd3*e$^{-/-}$ T-ALL had a late cortical phenotype, characterized by the appearance of ISP8 and DP cells only at the leukemic stage. Transcriptome analysis of *Cd3*e$^{-/-}$ T-ALL also indicated that these cells resemble post-β-selection thymocytes, even in the absence of a functional pre-TCR. Through a systematic regulator analysis exploring ChIP-seq binding profiles, we found that E protein targets were down-regulated during leukemia progression, and confirmed that E protein levels were low to undetectable in leukemic cells. Finally, lowering E protein gene dosage was sufficient to bypass pre-TCR signalling and restore full penetrance, indicating that inhibition of E proteins by the pre-TCR is a major collaborating event in T-ALL.

Conclusion: Our results demonstrate that the pre-TCR/CD3 complex signalling and its downstream inhibition of E protein transcription factors - which is a pathway that operates at the β-selection checkpoint parallel to Notch1 signalling- determines disease agressivity and penetrance in SCL-LMO1 T-ALL. We showed that, in the context of defective pre-TCR/CD3 signalling (*Cd3e*$^{-/-}$ T-ALL), progression to leukemia mimics the events at the β-selection checkpoint, including the acquisition of a gene signature that resembles the proliferation signature induced at the β-selection checkpoint, as well as down-regulation of E proteins. These results shows that T-ALL onset depends on efficient passage at the β-selection checkpoint, and the elucidation of this critical pathway opens new opportunities for targeted therapy in T-ALL.

P22

Title: PP2A-B55 is required for stress resistance by targeting 4EBP in *Drosophila* and in cancer cells.

Authors: Peng Wang, Post PhD 2014-2016

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Keywords: PP2A-B55, translation, eIF4E, 4E-BP, stress resistance.

Background information: The protein phosphatase PP2A activity is commonly impaired in different types of leukemia. In animal models, restoration of PP2A activity selectively suppressed the survival and self – renewal leukemia cells but not quiescent hematopoietic stem cells. Remarkably, PP2A activating drugs have been demonstrated to antagonize leukemogenesis both *in vitro* and *in vivo*. On the other hand, the deregulation of cap-dependent translational factor eIF4E/4EBP1 is frequently described in many human cancers, including hematologic malignancies. PP2A may dephosphorylate and activate 4EBP1, which is crucial inhibitor of eIF4E-dependent translation. However, the precise molecular mechanism of PP2A-4EBP1 pathway is still unclear. Moreover, more than 70 PP2A holoenzymes can be assembled by combination of different subunit forms, which have the different substrate specificity. Therefore, further investigation of the precise molecular mechanism of PP2A–4EBP1 pathway and identification of the specific isoform of PP2A which activates 4EBP1 are necessary for better understanding of the translational control machinery in leukemia.

Purpose of the study: This work aims at identification of the specific isoform of PP2A dephosphorylating 4EBP, and their interactions will subsequently be investigated in leukemia cells. I expect that my work could provide new and more specific leukemia therapeutic targets.

Methods: All PP2A regulatory subunits, which confer PP2A holoenzyme substrate specificity, will be cloned and expressed in cells. Then, physical association between 4EBP and specific regulatory subunit of PP2A will be examined by co-immunoprecipitation (co-IP) assay. Subsequently, phosphatase assay will be performed to further investigate if identified specific isoform of PP2A from co-IPs can dephosphorylate 4EBP in vitro. Then, the functional study of these interactions will be performed in *Drosophila*, cancer cells and mouse by varying the expression of PP2A and 4EBP. If I can confirm that identified specific isoform of PP2A regulates cancer cells proliferation and viability by targeting 4E-BP, I will explore the possibility of pharmaceutical application of that specific form of PP2A in leukemia.

Results: I found that PP2A-B55, but not PP2A-B56, can associate with 4EBP both in fly cells and mammal cells. Moreover, B55 knockdown leads to the hyperphosphorylation and degradation of 4EBP in fly cells and in human skin fibroblasts. However, in cancer cells, PP2A-B55 is uniquely required for 4EBP dephosphorylaiton and activation under stress conditions. Flies heterozygous for B55 mutant and B55 knockdown cancer cells are more sensible to nutritional stress. These results indicate that PP2A-B55 is required for stress resistance by targeting 4EBP in both flies and cancer cells. Further investigation of PP2A-B55 – 4EBP interaction in leukemia cells is in preparation.

Conclusion: My results indicate that B55 specific form of PP2A is required for 4EBP activation, which has been demonstrated to play crucial role in proliferation and viability of leukemia cells. Under normal conditions, PP2A-B55 – 4EBP pathway presents tumor suppressor activity by blocking eIF4e dependent translation and proliferation. Whereas, under starvation conditions, PP2A-B55 has pro-tumorigenic function by promoting tumor adaptation to metabolic stress. Thus, further study of the precise molecular mechanism linking PP2A-B55 with 4E-BP can contribute to a better understanding of the translational control machinery in leukemia.

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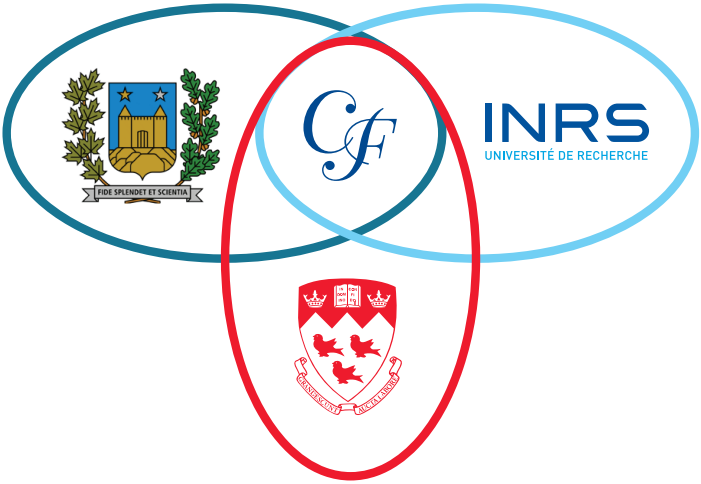
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