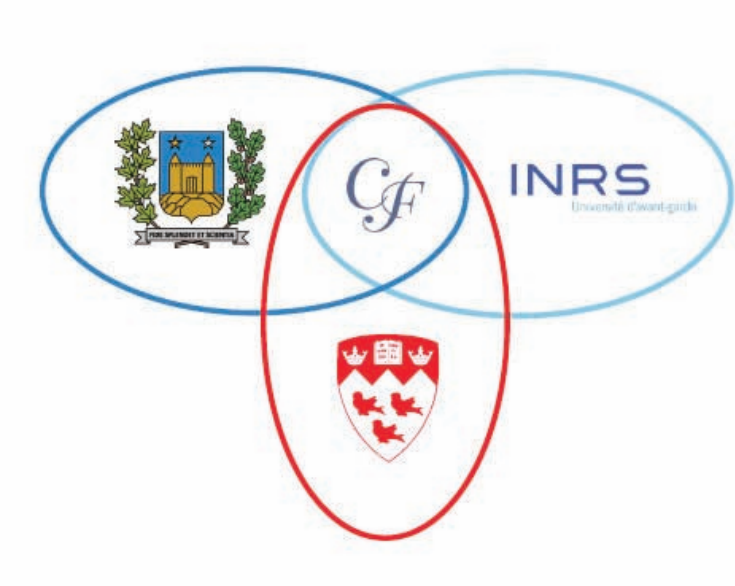
Cole Foundation
Research Celebration Day
May 14, 2010



la journée de la Fondation Cole
pour la recherche
14 mai 2010

Program/Programme

9:30 - 10:45 AM	Poster Set-up Période d'affichage
10:00 - 10:30 AM	Round table (new fellows) Table ronde (nouveaux boursiers) *Holmes Hall, Faculty of Medcine* *Deanery, 3605 de la montagne*
10:45 AM - 12:00 PM	Poster Session Part 1 Session d'affiches partie 1
12:00 - 1:00 PM	Lunch/ Dîner
12:30 - 1:45 PM	Poster Session Part 2 Session d'affiches partie 2
2:00 - 3:15 PM	Lecture/ Conférence *Martin Amphitheater*
Welcome/Bienvenue	*Barry Cole* *President/Pr*ésident *Cole Foundation*
Introduction/Introduction	*Michel Tremblay* *Director/ Directeur* *Goodman Cancer Research Centre*
Keynote Speaker/ *Conferencier invite*	*John E. Dick, Ph D, FRSC* *Senior Scientist, Division of Cellular and Molecular Biology, Toronto General Research Institute*
3:15 - 3:30 PM	New fellows and announcement of prizes Nouveaux boursiers et remise des prix
3:30 PM	Reception Réception

The 2010 - 2012 Cole Foundation Fellows
Les boursiers de recherche de la Fondation Cole 2010 - 2012

McGill University

Kimberley Doucette, Masters program
Supervisor: Claire Enfante-Rivard, Department of Epidemiology, Biostatistics and Occupational Health
Project title: The role of genetic and environmental determinants and their interaction in childhood leukemia
Our study will pursue the investigation of genetic susceptibility, environmental determinants, and their interaction, in children with acute lymphoblastic leukemia (ALL). Identification and confirmation of previously identified genetic variants and environmental factors shown to influence the incidence of ALL will be analyzed in data pooled from our and other large studies carried out worldwide.

Jessica Nichol, PhD program
Supervisor: Wilson Miller, Jr., Experimental Medicine, Lady Davis Institute
Project title: A Novel Mechanism of Retinoic Acid Resistance in Leukemia: Characterization of the Topoisomerase II Beta Co-Repressor Complex
My thesis project addresses important questions regarding how leukemia cells respond and/or develop resistance to retinoic acid (RA), a vitamin A derivative used to treat leukemia in the clinic. My research objective is to elucidate the mechanisms of aberrantly high protein levels observed in RA-resistant cells.

Torsten Nielsen, PhD program
Supervisor: Wilson Miller, Jr., Experimental Medicine, Lady Davis Institute
Project title: Predictive molecular markers of response to histone deacetylase inhibitors, with and without rituximab, in diffuse large B-cell lymphoma; a molecular approach with clinical correlates
Our research focuses on developing molecular markers predicting response to treatment with the drug class histone deacetylase inhibitors (HDACi) in patients with diffuse large B-cell lymphoma using cell line models as well as pre- and post-exposure patient samples. We are also investigating rational drug combination partners for HDACi treatment.

David X. Q. Wang, Masters program
Supervisor: Josée Dostie, Goodman Cancer Centre
Project Title: Role of non-coding RNAs in regulation of HoxA transcription factors in childhood mixed lineage leukemia.
We are interested in the three-dimensional architecture of the HoxA gene locus upon dysregulation by aberrant chimeric transcription factors in childhood mixed lineage leukemia. Our research also investigates functional non-coding RNAs transcribed from the HoxA locus and their effects in gene regulation.

Ryan Weist, PhD program
Supervisor, Xiang-Jian Yang, Goodman Cancer Centre
Project title: The kinase-HDAC-MEF2 signaling axis in normal and leukemic cellular programs
Post-translational modifications are important for controlling MEF2 function in hematopoietic and leukemic cellular programs. Accordingly, we have been examining the relationship between multisite modifications of normal as well as leukemic MEF2 proteins and a novel signaling cascade.

Université de Montréal

Aurélie Baguette, Post-PhD program
Supervisor: Katherine Borden, IRIC, Pathologie et biologie cellulaire
Project title: How the eIF4E-dependent mRNAs export pathway is regulated in normal and acute myeloid leukaemia specimens
This proposal focuses on the determination of molecular mechanisms that regulate the mRNA export function of eIF4E in normal and acute myeloid leukemia specimens. The characterization of these mechanisms should contribute to a better understanding of the ability of eIF4E to promote growth and survival of cancer cells.

Cédric Carli, Post-PhD program
Supervisor: Philippe Roux, IRIC, Pathologie et biologie cellulaire
Titre de projet: Régulation de Chk1 et du point de contrôle G2/M du cycle cellulaire par la voie de signalisation Ras/MAPK, et développement d'un traitement thérapeutique de la leucémie myéloïde
The signaling pathway Ras/MAPK and its substrate RSK are aberrantly activated in 75% of acute myeloid leukemia. We have identified the cell cycle regulator Chk1 as a new substrate of RSK and plan to characterize this interaction as it could be of crucial interest for future design of leukemia therapies.

Benjamin Neveu, Masters program
Supervisor: Daniel Sinnett, Pédiatrie, CHU Sainte-Justine
Titre de projet: Caractérisation des gènes cibles du facteur de transcription ETV6. L'élaboration de son réseau de régulation.
Le facteur de transcription ETV6 semble être un acteur important dans l'apparition et le développement de la leucémie lymphoblastique aiguë. L'identification de ses cibles transcriptionelles et l'élaboration de son réseau de régulation permettront de mieux comprendre l'impact d'ETV6 dans cette maladie.

Alexandre Orthwein, PhD program
Supervisor: Javier Di Noia, Faculté de Médecine, Université de Montréal
Project title: Dependence of AID stability on molecular chaperones: Implication for leukemia/lymphoma development and treatment.
We are studying AID, a central enzyme in the immune system, which can also predispose to lymphoma, leukemia and perhaps other cancers. We aim to understand AID regulation to potentially prevent its role in the development of cancer.

Danielle de Verteuil, PhD program
Supervisor: Claude Perreault, IRIC, Médecine
Titre de projet: Impact de l'immunoprotéasome sur le transcriptome des cellules dendritiques
L'immunoprotéasome, complexe surtout connu pour sa fonction dans la dégradation protéique et dans la production d'antigènes du soi, est exprimé spécifiquement dans les cellules immunitaires et plusieurs cancers hématologiques. Nous nous intéressons à une nouvelle fonction de l'immunoprotéasome sur la transcription, et cherchons à comprendre comment cela module la transformation néoplasique.

Krystel Vincent, Masters program
Supervisor, Claude Perreault, IRIC, Médecine
Titre de projet: La perturbation de mTOR change la composition de l'immunopeptidome du CMH I dans les cellules cancéreuses
La présentation d'antigènes à la surface cellulaire pour la surveillance immunitaire est régulée par la synthèse et la dégradation protéique. D'ailleurs, la voie de signalisation mTOR joue un rôle crucial dans ces deux processus. Nous désirons évaluer comment une perturbation de cette voie affecte la génération des antigènes de surface.

The Cole Foundation

The Cole Foundation offers fellowships to clinical fellows, postdoctoral residents and graduate students in PhD and MSc 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 public announcement today, the Fellowship programme has supported over 60 researchers in laboratories and hospitals situated in the Greater Montreal area through collaborations with l'Institut national de la recherche scientifique - Institut Armand-Frappier; l'Université de Montréal; and McGill University. Over $2 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. The Cole Foundation Fellowship Programme is just one example of this new and important activity.

The Cole Foundation:
Barry Cole – President
John Moran – Secretary/Treasurer
David Laidley – Board Member
Anne Lewis – Board Member
Bruce McNiven – Board Member
Bill Ridley – Board Member
Dr. Guy Rouleau – Board Member
Dr. Sheila Horn Bisaillon – Advisor
Dr. Maurice McGregor - Advisor

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 et de maîtrise ès sciences. Avec l'annonce publique d'aujourd'hui, le programme de bourses aide financièrement plus que 60 chercheurs répartis dans des laboratoires et des hôpitaux de l'agglomération montréalaise, à la faveur de collaborations avec l'Institut national de la recherche scientifique – Institut Armand-Frappier; l'Université de Montréal; et l'Université McGill. Plus de deux millions de dollars y seront 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'œuvre 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. Le programme de bourses de la Fondation Cole n'est qu'un exemple de cette nouvelle activité importante.

La Fondation Cole :
Barry Cole – Président
John Moran – Secrétaire/Trésorier
David Laidley – Membre du conseil d'administration
Anne Lewis – Membre du conseil d'administration
Bruce McNiven – Membre du conseil d'administration
Bill Ridley – Membre du conseil d'administration
Dr Guy Rouleau – Membre du conseil d'administration
Dre Sheila Horn Bisaillon – Conseillère
Dr Maurice McGregor - Conseiller

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

Presenters

Barakat, Stéphane
Bourgey, Mathieu
Bourgeois-Daigneault, Marie-Claude
Cargnello, Marie
Caron Étienne
Carrière-Pazat, Audrey
Cencic, Regina
Delgoshaie, Neda
Fournier, Marilaine
Frémon, Christophe
Guiguère, Amélie
Giroux, Martin
Hulea, Laura
Kahndanpour, Cyrus
Koonpaew, Surapong
Mills, John
Nichol, Jessica N
Petruccelli, Luca
Robilotta, Alexia
Rojas-Sutterlin, Shanti
Shirokova, Elena
Sincennes, Marie-Claude
Tawar, Urmila
Tremblay, Mathieu
Wilhem, Brian
Yu, Helen

Poster List

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PO2	Bourgey, Mathieu	A NOVEL STATISTICAL APPROACH TO MEASURE MATING ASYMMETRY: APPLICATION IN HUMAN POPULATIONS
PO3	Bourgeois-Daigneault, Marie-Claude	IMPLICATION OF MARCH FAMILY MEMBERS IN DEVELOPMENT AND PROGRESSION OF ACUTE TYPE LEUKEMIAS
PO4	Cargnello, Marie	REGULATION OF 4E-T FUNCTION, THE EIF-4E TRANSPORTER, BY GROWTH FACTORS AND ONCOGENES.
PO5	Caron, Étienne	IMPRINT OF RAPAMYCIN-MEDIATED mTOR INHIBITION IN THE MHC I IMMUNOPEPTIDOME

PO6	Carrière-Pazat, Audrey *(not present)*	DETERMINING THE ROLE OF THE ONCOGENIC RAS/MAPK PATHWAY AS AN UPSTREAM REGULATOR OF MTOR.
PO7	Cencic, Regina	REVERSING CHEMORESISTANCE WITH INHIBITORS OF EIF4E:EIF4G INTERACTION
PO8	Delgoshaie, Neda *(not present)*	REGULATION OF HISTONE H3 LYSINE 56 ACETYLATION DURING THE CELL CYCLE AND THE DNA DAMAGE RESPONSE
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PO10	Frémon, Christophe	GENETIC AND PHOSPHOPROTEOMIC ANALYSIS OF THE SPECIFIC FUNCTIONS OF ERK1 AND ERK2 MAP KINASES IN CELL PROLIFERATION
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PO12	Giroux, Martin	DONOR SMAD3 DEFICIENCY USES LETHAL GRAFT-VERSUS-HOST DISEASE BY INCREASING TH1 SKEWING AS WELL AS GRANULOCYTE EXPANSION AND ACTIVITY
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PO14	Kahndanpour, Cyrus	GROWTH FACTOR INDEPENDENCE 1 (GFI1) IS REQUIRED FOR THE INITIATION, PROGRESSION AND TRANSPLANTABILITY OF LYMPHOID LEUKEMIA AND REPRESENTS A POTENTIAL TARGET FOR A NEW THERAPEUTIC APPROACH
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PO20	Rojas-Sutterlin, Shanti	E2A AND SCL ARE FUNCTIONAL PARTNERS THAT MAINTAIN THE POOL OF ADULT HEMATOPOIETIC STEM CELLS
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PO24	Tremblay, Mathieu	MODELING T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA INDUCED BY THE SCL AND LMO1 ONCO GENES
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PO26	Yu, Helen *(not present)*	ROLE OF BAP1 TRANSCRIPTIONAL COMPLEX IN LEUKEMIA

PO1

Title: ETO2 controls hematopoietic stem cell expansion via the Nervy homology domain 1.

Authors: S. Barakat, J. Lambert, G. Sauvageau, T. Hoang

Affiliation: Trang Hoang, Université de Montréal, IRIC

Keywords: Hematopoietic stem cell, self-renewal, leukemia

Background information: Hematopoietic stem cells (HSCs) self-renew in vivo to sustain the life-long production of blood cells. However, HSCs rapidly decline in culture. Although some of the genes controlling self-renewal have been identified, overall, mechanisms governing HSC maintenance and self-renewal remain to be elucidated. We recently show that SCL (stem cell leukemia), a bHLH transcription factor, controls HSC quiescence and long term competence. Using a proteomics approach to identify components of the SCL complex in erythroid cells, we and others identified the ETO2 co-repressor that limits the activity of the SCL complex via direct interaction with the E2A transcription factor. ETO2/CBFA2T3 is highly homologous to ETO/CBFA2T1 and both are translocation partners for AML1.

Purpose of the study: We propose to identify novel functions of ETO2 in the regulation of HSCs expansion.

Methods: Eto2 expression was defined by qRT-PCR of purified long term -HSC (LT-HSCs, CD34-Kit+Sca+Lin- or CD150+CD48-Kit+Sca+Lin-), short-term HSC (CD34+Flt3-Kit+Sca+Lin-) and lympho-myeloid progenitors (LMPP, CD34+Flt3+Kit+Sca+Lin-) and protein levels assessed by flow cytometry analysis of these populations. Eto2 functional studies include overexpression using the MSCV vectors, and RNA interference using lentiviral delivery. Transduced HSCs were analysed by transplantation.

Results: We initially found that ETO2 is highly expressed in populations of cells enriched in ST-HSCs and LMPP, and at lower levels in LT-HSCs, correlating with ETO2 protein levels by flow cytometry. Next, we show that shRNAs directed against ETO2 knock down ET02 protein levels in the KSL population, causing a ten-fold decrease in this population after transplantation, associated with reduced short-term and long-term reconstitution in mice. Conversely, ectopic ETO2 expression induces a 100 fold expansion of LT-HSCs in vivo in transplanted mice associated with differentiation blockade in all lineages, suggesting that ETO2 overexpression overcomes the mechanisms that limit HSC expansion in vivo. Furthermore, this expansion is abrogated when NHR1 domain of ETO2 is deleted. Surprisingly, overexpression of ETO2 but not of the NHR1 mutant results in a 1500 fold expansion of the KSL and KSL150+48- in vitro over 4 weeks. At the cellular level, we found that ETO2 facilitates the G0/G1 as well as G1/S transitions in LT-HSCs, whereas the NHR1 mutant causes G1/S blockade associated with apoptosis in the KSL population.

Conclusion: In conclusion, we show that ETO2 is highly expressed in ST-HSCs and lymphoid progenitors, and controls their expansion by regulating cell cycle entry both at the G0/G1 and the G1-S transitions. In addition, ETO2 overexpression converts the self-rnewal of maintenance into self-renewal of expansion in LT-HSCs.

PO2

Title: A novel statistical approach to measure mating asymmetry: application in human populations

Authors: Mathieu Bourgey, Daniel Sinnett

Affiliation: Hematology-Oncology Department, Research center of the CHU Sainte-Justine hospital, University of Montreal, Montreal, PQ, Canada

Keywords: Acute lymphoblastic leukemia, feto-maternal effect, mating asymmetry

Background: Acute lymphoblastic leukemia (ALL) is the most common form of cancer in children. Since decades genetic analysis have detected numerous loci associated with ALL. But these loci account for only a small fraction of the genetic variability of this disease. To find the missing heritability in ALL, we have focused on the study of feto-maternal genetic interaction. It is now accepted that a mother can influence her offspring's risk of disease through the effects of her genes. We have shown that feto-maternal gene interaction occurs in ALL (Healy and Bourgey et al. 2010). In addition, we demonstrated that mating asymmetry (MA) is a major confounding factor to detect these effects. MA can lead to distortions in the distribution of alleles among case-mothers and create spurious maternal associations.

Purpose of the study: We want to evaluate and understand the pattern of mating asymmetry in human populations.

Methods: Mating symmetry refers to the hypothesis that for a parental genotype pair, the frequency in the population for a given mother-father genotype assignment is the same as for the reverse father-mother assignment. We propose a new quantification estimator for mating asymmetry (MaS) to determine the significance of asymmetry at a given locus. To achieve a better understanding of the dynamics of mating asymmetry in human populations we carried out a genome-wide evaluation of MA using mate-pairs of European and African ancestry from the International HapMap Project. Additionally we performed a gene ontology analysis and several simulation studies to explain the pattern of mating asymmetry observed in our data.

Results: HapMap data shows a similar pattern of MA in European and in African populations with some high peaks of asymmetry spread in several places in the genome. Simulation study exclude the random origin (ascertainment or type I error) of the asymmetry. The genic location of peaks and the gene ontology analysis indicate that biological processes do not influence the mating asymmetry pattern. Analysis of an artificial population based on a random origin of mate gives evidence for a possible population effect that can participate to the observed asymmetry. Finally, we show that levels of mating asymmetry observed in HapMap are sufficient to create spurious maternal associations.

Conclusions: Our study showed how it is possible to measure mating asymmetry in human. The levels of mating asymmetry observed in the HapMap data are in excess of random levels. This finding indicates that we should take it into account mating asymmetry in association studies in order to avoid false positive maternal and feto-maternal associations. Dealing with spurious association could dramatically slow down the understanding of the genetic component of a complex disease such as ALL.

P03

Title: Implication of MARCH family members in development and progression of acute type leukemias

Authors: Marie-Claude Bourgeois-Daigneault, Jacques Thibodeau

Affiliations : Jacques Thibodeau, Département de microbiologie et immunologie, Université de Montréal

Keywords: MARCH1, MARCH8, ubiquitination, IL-10, IL-6

Background information: Leukemia is caused by the uncontrolled proliferation of lymphoid or myeloid cells. Multiple strategies are responsible for the immune evasion of these cells. One of which is the down-modulation of surface MHCII molecules. IL-10 and IL-6 are involved in this phenomenon and thus, high levels of these cytokines are unfavorable prognostis factors of leukemia. In our latest study, we identified MARCH family members as key players in the IL-10-induced down-regulation of MHCII.

Purpose of the study: The objective of this research project was to investigate the role of MARCH family members, especially MARCH1 and MARCH8, in the development and proliferation of acute myeloid leukemia.

Methods: Quantitative real-time PCR was used to monitor the mRNA levels of MARCHs in primary human monocytes and immatures and matures B cells. The induction of MARCHs was also investigated for cells stimulated with IL-6 or IL-10. The implication of MARCH1 on the STAT3 and NF-kB DNA binding ability was investigated by luciferase assays. Moreover, immunoprecipitations, confocal microscopy and FRET were performed to characterize the mechanisms by which MARCH1 interferes with the signal transduction of IL-10 and IL-6.

Results: Our results showed an induction of MARCH1 and MARCH8 in primary human monocytes stimulated by IL-10 and IL-6 respectively. Also, these two MARCH family members showed higher mRNA levels in unstimulated B cells compared to CD40-activated B cells. Luciferase assays showed a diminished DNA binding ability of STAT3 and NF-kb in the presence of MARCH1 and 8. Finally, immunoprecipitations and FRET showed a direct interaction between STAT3 and MARCH1 and 8 and a vesicular relocalisation of STAT3 in the presence of MARCH1.

Conclusions: Our results showed a modulation of two MARCH family members during the activation of B cells. Also, MARCH1 and 8 were showed to be implicated in the signal transduction by IL-10 and IL-6, two cytokines closely related to the prognostis of leukemia development. Our data suggests a model where MARCH1 and 8 would inhibit the signal transduction by these two cytokines by relocating and sequestering STAT3 on vesicles thus preventing it from binding DNA and inducing gene expression.

PO4

Title: Regulation of 4E-T function, the eIF-4E transporter, by growth factors and oncogenes.

Authors: Marie Cargnello, Philippe P Roux

Affiliation: Department of Pathology and Cell Biology, Institute for Research in Immunology and Cancer (IRIC), Université de Montréal.

Keywords: Oncogene, Cell signalling, Translation of mRNA, 4E-T

Background: The eukaryotic initiation factor 4E (eIF-4E) is a key regulator of mRNA fate that is overexpressed in numerous human cancers including hematological malignancies such as leukemia. eIF-4E is regulated in normal cells through its interaction with different partners, such as its transporter 4E-T. 4E-T was originally described as a nucleocytoplasmic shuttling protein that mediates nuclear import of eIF4E. Since, it has been proposed that 4E-T participates to the assembly of processing bodies (P-bodies), which are discrete foci in the cytoplasm where takes place mRNA storage and degradation. 4E-T may also act as a translational repressor by sequestrating eIF4E to P-bodies. Whether 4E-T is regulated by post-translational modification remains unknown. Preliminary results from our group indicate that 4E-T becomes phosphorylated in response to several growth factors and oncogenes.

Purpose of the study: Our main objective is to identify and characterize signaling events that lead to 4E-T phosphorylation. Further, we want to determine the role of specific phosphorylation events with regards to the oncogenic properties of eIF-4E.

Methods: To detect the phosphorylation of 4E-T, we mainly used mobility shift assays and a phospho-motif antibody recognizing typical MAPK substrates. We used pharmacological inhibitors to study the involvement of signaling cascades in 4E-T phosphorylation. Finally we used site-directed mutagenesis and mass spectrometry to identify specific 4E-T phosphorylation sites.

Results: We demonstrate that activation of the Ras/MAPK pathway promotes the phosphorylation of 4E-T. Moreover, oncogenic alterations of this pathway also induce strong phosphorylation of 4E-T. Using phosphorylation site mutants of 4E-T, we identify two residues located in a basophilic motif, Thr257 and Ser259, that are phosphorylated in response to Ras/MAPK pathway activation.

Conclusion: We identified 4E-T as a new substrate of the Ras/MAPK pathway and identified two important phosphorylation sites. In the future, we hope to elucidate the impact of 4E-T phosphorylation on its biological functions that are linked to the eIF-4E oncogene. Since an eIF-4E inhibitor is currently being tested in a clinical trial for the treatment of AML, these experiments will demonstrate whether combined pharmacological inhibition of both Ras/MAPK and eIF4E represents a suitable strategy for therapeutic treatment of AML.

PO5

Title: IMPRINT OF RAPAMYCIN-MEDIATED mTOR INHIBITION IN THE MHC I IMMUNOPEPTIDOME

Authors: Étienne Caron1,5, Marie-Hélène Fortier1,2, Krystel Vincent1,5, Jean-Philippe Laverdure1, Alexandre Bramoullé1,4, Dariel Ashton-Beaucage1,3, Mathieu Courcelles1,4, Sébastien Lemieux1, Philippe Roux1,3, Pierre Thibault1,2, Claude Perreault1,5.

Affiliation: Institute for Research in Immunology and Cancer1, Department of Chemistry2, Department of Pathology and cell biology3, Department of Biochemistry4, Department of Medicine5, University of Montreal, Montreal, Quebec, Canada

Keywords: MHC I-peptide, rapamycin, mTOR, mass spectrometry, transcriptome.

Background: Cell surface MHC I molecules are associated with self peptides that are collectively referred to as the self MHC I immunopeptidome (sMII). Importantly, the sMII is molded by transcript levels, mRNA translation and protein degradation. Although we know very little on the global composition of the sMII, changes in the sMII might virtually reflect any intracellular biological processes in response to pharmacological perturbations. In this regard, rapamycin was initially found to possess anti-tumor properties by inhibiting mTOR, which is one of the most frequently amplified oncogenic pathway. However, most recent observations suggest that rapamycin-mediated mTOR inhibition impinges on the regulation of different feedback loops that may increase cell survival and may contribute to rapamycin resistance.

Purpose of the study: Our first objective is to evaluate how rapamycin-mediated mTOR inhibition impacts on the global composition of the sMII. Our second objective is to evaluate to what extent changes in the sMII reflect specific intracellular biological processes in response to rapamycin perturbation.

Methods: By using a novel high-throughput mass spectrometry approach, we quantified the dynamic expression profile of 425 MHC I-associated peptides from rapamycin-treated EL4 lymphoblastoma cells. By using DNA microarrays, we also analyzed transcriptomic changes upon rapamycin treatment. In addition, we developed a network-based functional association matrix to statistically correlate immunopeptidome and transcriptome mapping data. Finally, we evaluated the regulation of peptide source genes by qPCR, western blot, and flow cytometry.

Results: Remarkably, we observed that 80% of MHC I peptides were progressively overexpressed in response to rapamycin. By integrating immunopeptidomic data, transcriptomic data, and functional associations from databases, we demonstrated that most differentially expressed MHC I peptides are encoded by source genes that were functionally related to both the transcriptomic signature and the mTOR signaling network. Moreover, our data showed that most peptide source genes were regulated at the posttranscriptional level within gene regulatory subnetworks that were perturbed in response to rapamycin.

Conclusions: Here, we conclude that rapamycin-mediated mTOR inhibition drastically influences the composition of the sMII. Importantly, our results show that changes in the sMII are not random but reflect specific intracellular biological processes in response to rapamycin perturbation. Moreover, we propose that analysis of sMII-derived data provides a unique perspective on protein economy in general and on the cellular response to rapamycin in particular.

PO6 *(not present)*

Title: Determining the role of the oncogenic Ras/MAPK pathway as an upstream regulator of mTOR.

Authors: Audrey CARRIERE-PAZAT, Yves ROMEO, Philippe P. ROUX.

Affiliation: Philippe P. ROUX, Institute for Research in Immunology and Cancer (IRIC), Université de Montréal.

Keywords: Cell Signaling, mTOR, Ras/MAPK, phosphorylation, Raptor.

Background information: The mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase that integrates signals from nutrients, energy sufficiency and growth factors to regulate cell growth. The mTOR pathway is aberrantly activated in many types of cancers including hematological malignancies such as acute myeloid leukemia. However, the molecular mechanisms at the origin of mTOR hyperactivation remain elusive. Some evidence suggest that the oncogenic Ras/MAPK pathway controls mTOR activity but the direct molecular mechanisms linking both signaling pathways remain to be further determined. We have very recently demonstrated that Raptor, an essential scaffolding protein of the mTOR complex 1 (mTORC1), was strongly phosphorylated following activation of the Ras/MAPK pathway through the p90 ribosomal S6 kinase (RSK), promoting mTORC1 activity and thereby demonstrating a direct molecular link between both pathways.

Purpose of the study: Our objective is to further explore how the Ras/MAPK pathway affects the phosphorylation status of Raptor and the consequences on mTOR activity. More precisely, we are currently investigating the role of the extracellular signal-regulated kinases ERK1/2, as new kinases phosphorylating Raptor.

Methods: To detect Raptor phosphorylation, we used a phospho-motif antibody that specifically recognizes proline-directed consensus sites typically found in ERK1/2 substrates. To demonstrate the involvement of ERK1/2 in Raptor phosphorylation, we used complementary strategies such as loss of function (pharmacological inhibitors, RNA interference and overexpression of dominant negative forms of ERK) and gain of function (overexpression of oncogenic versions of components of the Ras/MAPK pathway) experiments. To detect the precise phosphorylation sites, we performed quantitative mass spectrometry and site-directed mutagenesis. Protein-protein interaction was assessed by co-immunoprecipitation experiments and mTORC1 activity by in vitro kinase assays.

Results: We have shown that Raptor becomes highly phosphorylated on proline-directed sites following mitogen-induced activation of the Ras/MAPK pathway. Moreover, expression of oncogenic forms of Ras and MEK that elevate mTOR activity induced strong and constitutive phosphorylation of Raptor on these consensus sites. We found that ERK1 and ERK2 are required for Raptor phosphorylation in cells and directly phosphorylate Raptor in vitro. Moreover, we demonstrated that ERK and Raptor interact with each other, suggesting that ERK could be part of the mTORC1 complex. We precisely identified three proline-directed sites (Ser8, Ser696 and Ser863), which are directly phosphorylated by ERK1/2. mTORC1 kinase assays from cells expressing phosphorylation-deficient alleles of Raptor revealed that phosphorylation of the three sites promotes mTORC1 kinase activity, suggesting that ERK1/2-dependent phosphorylation of Raptor positively regulates mTORC1 signaling.

Conclusions: Unraveling signal transduction pathways at the origin of mTOR hyperactivation in cancer cells is of crucial interest as it may provide key additional targets for therapy. Our data reveals a new molecular link by which the Ras/MAPK pathway controls mTORC1 activity, through ERK1/2-mediated phosphorylation of Raptor.

PO7

Title: Reversing chemoresistance with inhibitors of eIF4E:eIF4G interaction

Authors: Regina Cencic1, Marilyn Carrier1, Francis Robert1 and Jerry Pelletier1,2

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Keywords: eIF4F, translation initiation, synergy, chemotherapy

Background information: The PI3K/Akt/mTOR signaling pathway is frequently activated in many cancer cells. mTOR is a regulator of translation initiation and its stimulation leads to increases in protein synthesis. The recruitment of the 40S ribosomal subunit and associated factors to the mRNA during translation initiation is highly regulated by eukaryotic initiation factor (eIF) 4F, a heterotrimeric protein complex. The translation initiation factor eIF4E is the least abundant factor of the eIF4F complex and has been shown to be overexpressed in many human cancers. Small molecule inhibitors that affect eIF4F function would be highly valuable in assessing the potential of targeting cap-dependent translation as a chemotherapeutic tool.

Purpose of the study: Screen for inhibitors of the interaction of the eIF4E and eIF4G subunits of eIF4F and test their potential in a pre-clinical murine lymphoma model.

Methods: A TR (time resolved) -FRET (fluorescence resonance energy transfer) based HTS (high throughput screening) assay that monitors the eIF4E-eIF4G interaction of the eIF4F complex was performed and 269,000 small molecules tested for their potential to disrupt this interaction. Hits were investigated for their specificity of inhibiting cap-dependent translation. Two small molecules were used for further studies on reversing chemoresistance in a murine Pten+/- Eμ-myc lymphoma model.

Results: Screening of 269,000 compounds led to 3 small molecule inhibitors of eIF4E:eIF4G interaction. These compounds inhibited cap-dependent in vitro translation and showed inhibition of eIF4F complex formation in vivo. Two of the compounds were tested for their ability to reverse chemoresistance in a pre-clinical murine lymphoma model. Both small molecules synergized with doxorubicin, leading to a significant delay in tumor relapse.

Conclusions: We found two inhibitors of eIF4E:eIF4G interaction that synergized with a standard-of-care agent in a pre-clinical murine lymphoma model. These are the first inhibitors of this interaction that have been shown to reverse chemoresistance in vivo. Since eIF4E has been shown to play an important role in tumorigenesis, these are promising results that inhibition of eIF4E:eIF4G interaction can indeed affect tumor development in vivo.

PO8 *(not present)*

Title: REGULATION OF HISTONE H3 LYSINE 56 ACETYLATION DURING THE CELL CYCLE AND THE DNA DAMAGE RESPONSE

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Keywords: histone, chromatin, cell cycle, DNA damage

Background: Histone H3 lysine 56 acetylation (H3 K56Ac) was first identified in the budding yeast S. cerevisiae, but has been subsequently detected in several other species including humans. A recent study on a variety of human tumors has demonstrated that H3 K56Ac is far more abundant in cancer cells than the neighboring normal cells. In S. cerevisiae and human cells, H3 K56Ac occurs transiently during normal passage through S-phase, but is maintained in response to DNA damage. Importantly, cells that cannot acetylate or deacetylate H3 K56 are acutely sensitive to several analogues of DNA damaging agents used in cancer chemotherapy.

Purpose of the study: The main objective of my project is to investigate the molecular mechanisms that underlie the regulation of H3 K56Ac during the cell cycle and in response to DNA damage, using S. cerevisiae as model.

Methods: I used several mutants that arrest the cell cycle at different stages in order to identify the window of time during which the majority of H3 K56Ac is removed from the genome. Moreover, I studied the regulation of H3 K56Ac in a mutant strain where DNA damage can be specifically induced at the ends of chromosomes, which are known as telomeres.

Results: In S. cerevisiae, H3 K56Ac peaks concomitant to DNA replication during S phase. I discovered that the bulk of H3 K56Ac is removed from the genome before entry into mitosis. Therefore, my results narrow the window of time for the removal of this genome-wide modification to late S or G2 phases of the cell cycle. Moreover, I found that telomeric DNA damage does not preclude removal of H3 K56Ac from most of the genome. However, someH3 K56Ac persists under these conditions, and my working hypothesis is that this residual H3K56Ac is localized at damaged telomeres.

Conclusions: My work revealed the specific window of time during which H3 K56Ac is removed from chromatin during a normal cell cycle. This information will be helpful to identify the master regulatory complexes that modulate this conserved modification during the cell cycle. In addition, my findings on the regulation of H3 K56Ac in response to telomeric DNA damage will help improve our understanding of the functions and regulation of this histone mark following DNA damage.

P09

Title: Overexpression of HOXA4 induces hematopoietic stem cell and myeloid progenitor expansion in vitro

Author: Marilaine Fournier, Janetta J. Bijl

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Keywords: Hematopoietic Stem Cell (HSC), Bone marrow (BM) transplantation, Hox Genes

Background information: A significant proportion patients suffering leukemia are candidates for hematopoietic stem cell transplantation. Low number of HSCs available limits this type of treatment. Homeobox (Hox) gene HOXB4 can induce HSC expansion in vivo and in vitro with preservation of their function. However, mutant mouse models showed that this gene is not required for the generation and function of HSCs. These contradictory observations might be explained by functional redundancy between Hox genes in hematopoiesis. Actually HOXA4 is expressed in HSC enriched foetal liver (E14.5) fractions at a 10-fold higher level than HOXB4 (Bijl, 2006). At this time point of ontogeny, HSCs are undergoing their principal expansion and thus suggests a potential role for HOXA4 in HSC self-renewal. This is further supported by the failure of HOXA4-/- HSCs to repopulate irradiated recipients under competitive conditions (unpublished) indicating a potential defect in HSC self-renewal.

Purpose of the study: Evaluation of HSCs expansion by HOXA4 in vitro.

Methods: To test whether HOXA4 expand HSCs in vitro we stably over-expressed HOXA4 in primary BM cells using MSCV-IRES-GFP based retroviral vectors. GFP positive cells were used to initiate our cultures. Overall growth was monitored for a period of 3 weeks. Colony forming cell (CFC) and competitive repopulation unit (CRU) assays were performed to determine the frequency of myeloid progenitors and HSC present in the culture respectively.

Results: HOXA4 BM cells grew significantly faster than control BM cells (n=3) over a three weeks culture period, suggesting a strong expansion of primitive hematopoietic cells to sustain the culture. Indeed, CFC assays showed that HOXA4 BM cells contained 239 more myeloid progenitors than CTRL cultures. These HOXA4 progenitors appeared more primitive than in control cultures indicating that the expansion might be at the level of HSCs. To evaluate whether the maintenance of the HOXA4 BM cultures was also the result of HSC expansion, we measured the frequency of HSCs at day 0 and day 6. We observed an expansion of HOXA4 HSCs with preservation of their properties as they contribute to both myeloid and lymphoid compartments. Preliminary data on HOXA4 chimeras also showed that the frequency of B cell progenitors in the BM was higher in HOXA4 cells compared to wt cells indicating a potential role for HOXA4 in the expansion of these progenitors

Conclusion: Thus, we show that HOXA4 can induce self-renewal of HSCs in vitro and is a very promising candidate for ex vivo expansion of HSCs. It still remains to be determined whether HOXA4 can expand B cell progenitors in vitro.

PO10

Title: GENETIC AND PHOSPHOPROTEOMIC ANALYSIS OF THE SPECIFIC FUNCTIONS OF ERK1 AND ERK2 MAP KINASES IN CELL PROLIFERATION.

Authors: Christophe Frémin, Sylvain Meloche.

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Keywords: ERK1/2, knock-in mouse, phosphoproteomics, cancer.

Background: The ERK1/2 MAP kinases (MAPK) signalling pathway is a highly conserved signalling module especially playing a central role in the control of cell proliferation. The activation of ERK1 and ERK2 kinases is catalyzed by MEK1 and MEK2 MAPK kinases, which are themselves phosphorylated and activated by different MAPKK kinases, including Raf isoforms. Activating mutations in Ras and Braf genes are found in 30% of human tumors and are often an early event in tumor progression. Besides, a constitutive activation of the signalling pathway has been reported in 40% of leukemia. Thus, the Ras-ERK1/2 is considered a particularly attractive target for the development of anti-cancer therapies.

Purpose of the study: However, several questions which are likely to have huge impact on the development of anti-tumor therapies remain unanswered. Of these, what are the individual contributions of ERK1 and ERK2 in the processes of cell proliferation and transformation? The analysis of mice deficient for each of Erk1 and Erk2 genes suggests that the two kinases may regulate unique features. This study aims to clarify the specific roles of the two kinases ERK1 and ERK2 by different approaches.

Methods: A. First, we are generating a knock-in strain of mice in which Erk2 gene is deleted and replaced by Erk1. To generate Erk2Erk1 knock-in mice, we constructed a targeting vector that upon homologous recombination replaces the coding sequence of Erk2 in exon 1 by the mouse ERK1 cDNA. Correctly targeted ES cells are then injected into C57BL/6 blastocysts to generate the knock-in mouse.
B. Secondly, a phosphoproteomic analysis is currently conducted on primary murine embryonic fibroblasts (MEFs) of wild-type, Erk1-/-, Erk2-/-, or Erk1-/-; Erk2-/- genotypes. To generate Erk2-/- MEFs, primary MEFs are isolated from Erk2flox/flox mice that we generated and the floxed allele is excised in vitro using a self-excising Cre-expressing retrovirus. Comparison of the phosphoproteomes will give us new insights about specific and common cell factors which depend on ERK1 and ERK2.

Results: A. By studying the phenotypes of Erk2Erk1 knock-in mice, we will ask whether ERK1 can functionally substitute for ERK2 and rescue the embryonic lethality caused by ERK2 deficiency. After having completed the construction of the homologous recombination vector, we screened the ES clones for the mutated Erk2 allele. Several positive clones were obtained, injected into blastocystes and we are now waiting for the knock-in mice.
B. Identification of a more complete list of substrates is critical to understanding how the ERK1/2 pathway works at the cellular level. After having developed the assay, particularly Erk2 gene excision with Cre-expressing retrovirus, we conducted the experiment that has been done in collaboration with Dr Thibault's lab. We identified several potential new ERK substrates that are currently being subjected to validation using standard biochemical and cellular assays.

Conclusion: This project should give new insights into the regulation of the MAPK pathway by precising the potential overlapping or specific roles of ERK1 and ERK2 kinases. A better understanding of the specificities of these kinases could help us to develop more targeted strategies to fight cancer, particularly in the treatment of leukemia and lymphoma.

PO11

Title: IDENTIFICATION AND CHARACTERIZATION OF CRYPTIC AND NOVEL RUNX1 FUSIONS IN ACUTE LEUKEMIA.

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Affiliation: Quebec Leukemia Cell Bank, Maisonneuve-Rosemont Hospital Research Center

Keywords: Leukemia; Translocation; RUNX1

Background information: Cytogenetic analysis is an essential tool in the management of patients with acute leukemia. Chromosomal translocations are important prognostic factors in this disease and often disrupt genes encoding transcription factors such as RUNX1 (chromosomal band 21q22). The RUNX1 gene is involved in the establishment of definitive hematopoiesis and is a key regulator of myeloid and lymphoid differentiation. RUNX1 is one of the most frequent rearranged genes in leukemia. It is fused to more than 20 different partner genes in various chromosomal translocations but a significant number of partners remains to be identified.

Purpose of the study: The aim of our project is to define the molecular mechanisms involved in the pathogenesis of acute leukemia by the characterization of novel or rare chromosomal translocations [t(1;21)(p22;q22), t(7;21)(p22;q22), t(8;21)(q23;q22), t(15;21)(q26;q22)] involving the RUNX1 gene.

Methods: Using fluorescent in situ hybridization with bacterial artificial chromosome clones and RT–PCR, we identified four fusion partners of RUNX1 in patients with acute leukemia. Sequencing of the fusion products allowed the identification of alternative transcripts and reciprocal fusion transcripts in leukemic cells. Long distance inverse PCR and high fidelity PCR were used to clone genomic breakpoints of the t(15;21) and t(7;21) translocations. Quantitative PCR and/or RT-PCR studies were performed to quantify or detect USP42, CLCA2 or SLCO3A1 gene expression in normal hematopoietic cells and in leukemic blasts.

Results: We cloned novel (CLCA2, 1p22.3) and rare (USP42, 7p22.1; TRPS1, 8q23.3) partner genes of RUNX1 in acute myeloid leukemia. In-frame fusion transcripts of RUNX1-USP42 and RUNX1-TRPS1 (and reciprocal fusion transcripts) were detected in t(7;21) and t(8;21) respectively. RUNX1-CLCA2 out-of-frame fusion transcripts were identified in t(1;21). Interestingly, t(1;21) and t(8;21) were associated with therapy-related leukemias. No fusion transcripts were detected in the Philadelphia chromosome-positive biphenotypic acute leukemia with a novel t(15;21). However, a deletion of the SLCO3A1 gene (15q26) was observed. Expression studies in leukemic cells revealed USP42 overexpression in t(7;21) cells. Moreover, genomic analyses of t(7;21) breakpoints revealed features of the non-homologous end joining repair mechanism, suggesting its involvement in the genesis of this translocation.

Conclusion: RUNX1-USP42 and RUNX1-TRPS1 fusions generate putative chimeric proteins with potential aberrant transcriptional activity. In contrast, RUNX1-CLCA2 fusions resulted in truncated proteins that could also be associated with a dominant negative effect over normal RUNX1. These abnormal RUNX1 proteins could contribute to the development of leukemia in our patients. The characterization of different RUNX1 translocations is essential to better understand the oncogenic mechanisms associated with these rearrangements. In addition, these studies contribute to identify new genes involved in leukemia and to correlate clinical findings with these novel genetic alterations.

PO12

Title: Donor Smad3 Deficiency Causes Lethal Graft-Versus-Host Disease by Increasing Th1 Skewing as well as Granulocyte Expansion and Activity.

Author: Martin Giroux, Jean-Sébastien Delisle, Simon-David Gauthier, Krista Heinonen, Julie Hinsinger, Billy Houde, Louis Gaboury, Marie-Josée Hébert and Claude Perreault.

Affiliation: Department of Medecine, IRIC, Université de Montréal, Québec, Canada.
Keywords: GVHD, TGF-β, AHCT, tolerance, bone marrow transplantation.

Background: allogenic haematopoietic cell transplantation (AHCT) is the treatment of choice for potentially fatal haematologic malignancies, such as leukemia. Graft versus Host Disease (GVHD), where grafted immune cells will attack the recipient to "reject" it, is by far the main barrier in AHCT. It is clear that GVHD is initiated by an early flare in donor T-cell responses. However, the occurrence and severity of GVHD are also determined by other unknown factors. Our laboratory recently showed in humans that higher expression of TGF-β pathway components (with SMAD3 being the best predictor) in donor cells were correlated to increased risk of GVHD, thus allowing us to define "dangerous donors" and "safe donors".

Purpose: to study the mechanisms behind the tolerance induced by the expression of SMAD3 and how its absence in donor cells can lead to fatal GVHD.

Methods: we used in vitro assays as well as a mouse model of AHCT with SMAD3 KO or WT littermate as donors and Balb.b as recipient. To evaluate specific organ pathology we used immuno-histochemistry and histology staining. Flow cytometry and microscopy allowed us to follow proliferation, activation and localization of different immune cell populations throughout the progression of disease.

Results: we show that SMAD3 deficiency converts a tolerant model of AHCT into lethal one, owing mainly to large bowel involvement. In order to confer a lethal phenotype, SMAD3 deficiency in both the T-cell and myeloid components of the graft are necessary. SMAD3 deficiency results in high CD4 T-cell Th1 skewing with elevated IFN secretion and increased granulocyte expansion and function. We further show physical and functional relationships between infiltrating SMAD3 deficient neutrophils and oxidative damage in the colon.

Conclusion: Our findings unveil an unexpected role for granulocytes in large bowel inflammation and highlight the particular relevance of the TGF-β/SMAD3 axis in colon GVHD, which constitutes the highest mortality and morbidity burden post-AHCT. We suggest that, in humans, inter-individual genetic variations in SMAD3 and other components of the TGF-β pathways will impact GVHD occurrence and thus the monitoring of their expression could be used to lead to better donor selection. Moreover, the gut is directly accessible to pharmacological agents. Therefore, the design of molecules that would locally stimulate the TGF-β/SMAD3 pathway, without having systemic effects, would dramatically reduce gastro-intestinal GVHD without interfering with, for example, systemic anti-leukemia responses.

PO13

Title: The role of Rev1 in the generation of point mutation in mammalian cells

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Keywords: REV1, point mutation, transcription factor, Gleevec, BCR-ABL

Background information: Cancer is a genetic disease that results from the accumulation of mutations that cause the activation of oncogenes and the inactivation of tumor suppressors. Mutations are changes in DNA. These changes may involve large rearrangements, like in the case of the Philadelphia chromosome in chronic myelogenous leukemia (CML), or single changes in the DNA sequence called point mutations. Research on CML leading to the development of the drug imatinib-mesylate (commercial name, Gleevec) represents a paradigm in the fight against cancer. Following treatment with imatinib, most CML patients in chronic phase are in remission for a long period of time. However, a major problem is that leukemic cells can develop resistance to imatinib, leading to relapse of the disease. Resistance to imatinib results most often from point mutations that prevent binding of imatinib to the protein that causes the disease, BCR-ABL.

Purpose of the study, Methods and Results: Until recently point mutations were believed to be produced by a passive mechanism involving rare mistakes made by replicating DNA polymerases and not corrected by mismatch DNA repair. We now know that most point mutations in fact are generated by an active process involving the replacement of a high-fidelity DNA polymerase with low-fidelity DNA polymerases capable of carrying translesion synthesis (TLS), ie. replication past certain DNA lesions. One of these polymerases, REV1, fulfils an essential function as a scaffolding protein that recruits several TLS polymerases to DNA. The C-terminal domain of REV1 can bind and recruit several TLS polymerases, while REV1 is itself recruited to specific genomic locations via protein-protein interactions that involve other domains of REV1. We have accumulated evidence showing that REV1 can be recruited to DNA following an interaction between its N-terminal BRCT domain and a transcription factor that is phosphorylated by a checkpoint kinase.

Interestingly, a point mutation in the yeast REV1 BRCT domain was found to cause a reduction in the rate of point mutations. This information led us to devise a strategy to reduce the rate of point mutations. We hypothesized that overexpression of a REV1 N-terminal fragment that includes the BRCT domain but not the C-terminal domain would function as a dominant-negative mutant that squelches the rate of mutations. To test this strategy in the context of chronic myelogenous leukemia, we have established in the laboratory a tissue culture model that recapitulates the acquisition of resistance to imatinib by leukemic cells. Using BCR-ABL transformed Ba/F3 leukemic cells, we found indeed that the frequency of resistance to imatinib is reduced on average 7-fold in cells overexpressing the REV1 N-terminal fragment.

Conclusion: In summary, our results provide additional evidence to the notion that mutagenesis is an active process in mammalian cells, like in bacteria and yeast. Furthermore, we have succeeded in identifying a novel therapeutic target: a protein that is required for the generation of imatinib-resistant cells, and whose function involves a well-defined protein domain against which small molecule inhibitors can be developed.

PO14

Title: Growth factor independence 1 (Gfi1) is required for the initiation, progression and transplantability of lymphoid leukemia and represents a potential target for a new therapeutic approach

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Keywords Gfi1, lymphoma, apoptosis, targeted therapy

Background information: Despite advances in treatment, a significant proportion (25%-60%) of all lymphoma patients still die due to tumor relapse, which warrants efforts to improve the current therapy.

Purpose of the study: Gfi1 is a transcriptional repressor with key functions in the development and activation of B and T-cells. Moreover, a constitutive expression of Gfi1 can elicit T-cell lymphoma in mouse models and Gfi1 is highly expressed in human T-ALL. These findings prompted us to investigate whether Gfi1 may be required for the initiation, maintenance and progression of T- and B-cell lymphoma and whether the ablation of Gfi1 might lead to a regression of lymphoid malignancies.

Methods: We have used mice, in which T –cell lymphoma was induced either by infection with the non-acute transforming Murine-Moloney- Leukemia-Virus (MMLV), by treatment with the carcinogen N-Ethyl-N-Nitrosourea, or by transplantation with bone marrow cells expressing the intracellular, oncogenic form of Notch. All three approaches induce malignant T-cell lymphoma in mice with high penetrance. As a model for B-cell lymphoma we used E□Myc transgenic mice, which develop malignant B-cell lymphoma with a high incidence and a short latency period. To investigate whether Gfi1 in required for the formation in the above models, we deleted the Gfi1 gene either consitituvely by crossing with Gfi1-/- mice or conditionally by introducing floxed Gfi1 alleles that can be excised upon activation of a Cre recombinase.

Results: We can show that Gfi1 deletion is counter-selected during the development of T-lymphoma in our mouse models and that loss of Gfi1 delays tumor formation. When Gfi1 was deleted after the tumor was established, we observed a significant tumor regression, suggesting that Gfi1 is required for T-lymphoma maintenance. In addition we show that Gfi1 is also an essential factor for maintaining B-cell lymphoma, although the development ca occur without Gfi1 in contrast to T-cell lymphoma. In a leukemia therapy model in mice we demonstrate that targeting Gfi1 enhances the efficacy of irradiation and stem cell transplantation. We show evidence that ablation of Gfi1 sensitizes cells to p53-dependent apoptosis and enables curing mice of B and T-cell lymphoma in clinically relevant models.

Conclusion: We present evidence that Gfi1 is the preferred pathway for T-ALL initiation and is required for the maintenance of T-ALL and B-cell lymphoma. In addition, we describe experiments that point to Gfi1 as a novel target for clinical intervention to treat lymphoid malignancies.

PO15

Title: FUNCTIONAL CHARACTERIZATION OF NUP98-HOXA9-INDUCED LEUKEMIA USING DROSOPHILA AND MOUSE MODELS

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Keywords: Chromatin remodeling factors, leukemia, NUP98-HOXA9

Background: The molecular events enabling the leukemogenic action of NUP98-HOXA9 in humans remain poorly characterized.

Purpose of the study: Our main objective is to use Drosophila as a model system to identify new functional partners of NUP98-HOXA9 by genetic screening and then test their mammalian counterparts in mouse leukemia models to verify their implication.

Methods and Results: We have recently completed a NUP98-HOXA9-dependent genetic screen where 100,000 progeny have been scored. This led to the isolation of over 1,200 recessive lethal lines that act either as suppressors or enhancers of a NUP98-HOXA9-dependent eye phenotype. Thus far, we have been able to catagorized these modifiers into 56 independent complementation groups. Ten complementation groups have recently been mapped to specific locations onto chromosome II by crossing mutant alleles from this group to the DrosDel deficiency kit collection. One of 10 complementation groups is allelic to the grainy head (grh) gene, which encodes a transcription factor involved in the regulation of organ development and stem cell differentiation. The relevant role of grh and other candidate genes in NUP98-HOXA9-induced leukemia will be further revealed in our murine leukemia models and human cell lines. We previously found that that NUP98-HOXA9 functionally interact with the TALE family transcription factor hth/Meis as well as with genes encoding components of the chromatin remodeling Brahma complex (osa, brm and mor). Remarkably, two of our complementation groups correspond to mutant alleles linked to the osa and hth loci. These findings provide compelling evidence for the ability of the screen to identify mutations in relevant genes. To further accelerate the discovery of NUP98-HOXA9 interacting partners, we also conduct second set of screen by co-expressing NUP98-HOXA9 and HTH in ey-flp/FO system. NUP98-HOXA9 and HTH collaboration produced large overgrowths in fly eyes. We currently utilize this collaboration model to obtain a number of transcription factors that could play an important role in regulation of NUP98-HOXA9/HTH collaboration. A collection of transgenic flies (obtained from Vienna Drosophila RNAi center) containing RNAi targeting genes of various components of signaling pathways will be specifically co-expressed with NUP98-HOXA9 and HTH in the clones. This current method previously allowed us to verify the importance of EXD (HOX co-factor in fly) in the collaboration.

Conclusions: We reasoned that some of these complementation groups correspond to genes encoding bona fide modulators of NUP98-HOXA9 whose function has also been conserved in mammalian cells. This current methods should give us a comprehensive overview of which signaling pathways might be involved in the NUP98-HOXA9/HTH collaboration and NUP98-HOXA9-induced leukemia.

PO16

Title: DESIGN AND IMPLEMENTATION OF A SHRNA-BASED SCREEN TO IDENTIFY TRANSLATION FACTORS ESSENTIAL TO MCL-1 SYNTHESIS.

Authors: John Mills, Abba Malina and Jerry Pelletier

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Keywords: ABT-737, Mcl-1, Translation, Apoptosis

Background: A common occurrence in relapsed and refractory hematological malignancies is the presence of a defective cell death mechanism. This defect is often mediated by overexpression of Bcl-2 family member proteins which block death signals from being executed. A promising new therapy, ABT-263 (orally available form of ABT-737), is currently in clinical trials for treatment of refractory hematological malignancies. This compound targets several Bcl-2 family members to re-activate cell death in drug resistance cancers. However, a resistance factor has been indentified, Mcl-1. Mcl-1 acts similarly to Bcl-2 but is not inhibited by ABT-737. Mcl-1 has an exquisitely short half-life (~15 min). Therefore, inhibition of Mcl-1 synthesis rapidly eliminates Mcl-1. We predict that blocking Mcl-1 protein synthesis will be an effective means to re-sensitize hematological cancers to ABT-737.

Purpose of the Study: Design and validate a model system for carrying out a focused shRNA library screen against translational targets to identify those essential to Mcl-1 synthesis. We anticipate inhibiting essential components of Mcl-1 synthesis will synergize with ABT-737 to induce cell death in refractory hematological malignancies.

Methods: We generated primary Eu-myc/Bcl2/p53-/- B-cell lymphomas. These lymphomas are refractory to a variety of standard of care therapies. We then tested their sensitivity to ABT-737, Cycloheximide (a protein synthesis inhibitor shown to inhibit Mcl-1 synthesis) or to both drugs in combination. To confirm that loss of Mcl-1 sensitized lymphomas to ABT-737, we introduced retroviral- based shRNA targeting either Luciferase or Mcl-1. We then monitored for selection against Mcl-1 shRNAs in our lymphoma cultures +/- ABT-737. We then used this assay to further validate that genetically targeting the translation apparatus in our model was sufficient to chemosensitize lymphomas to ABT-737 ex vivo.

Results: Cycloheximide was capable of sensitizing B-cell lymphomas to ABT-737. Alone neither agent was effective but together they acted syngerstically ex vivo. In addition, we confirmed that reducing Mcl-1 levels with an shRNA was capable of sensitizing B-cell lymphomas to ABT-737. We were able to recapitulate what was observed with cycloheximide using an shRNA against an essential ribosomal protein (rpL15). These pilot experiments establish screening conditions for us to now proceed to a focused shRNA screen to uncover synthetic lethal interactions with ABT-737.

Conclusions. Our preliminary results confirm this our system is functional. Inhibition of global protein synthesis syngerizes with ABT-737. Our positive control shRNA against Mcl-1 is eliminated only in the presence of ABT-737. Lastly, our model is able to identify protein synthesis targets that have potential to act synthetically lethal with ABT-737.

PO17

Title: Targeting PKCδ-mediated topoisomerase IIβ overexpression subverts the differentiation block in a retinoic acid-resistant APL cell line

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Keywords: acute promyelocytic leukemia, topoisomerase IIβ, PKCδ, differentiation

Background information: Acute promyelocytic leukemia (APL), is a subtype of acute myelogenous leukemia. At the genetic level, APL is characterized by a specific chromosomal rearrangement between the retinoic acid receptor alpha (RARA) and the promyelocytic leukemia (PML) genes. The resulting chimeric protein, PML-RARA, acts as a dominant negative inhibitor of normal retinoid receptor function. On the cellular level, the result is a block in granulocytic differentiation and an accumulation of myeloid progenitors. APL patients are treated with therapeutic doses of all-trans retinoic acid (RA), a vitamin A derivative that activates RARA and circumvents the differentiation block. Unfortunately, RA resistance develops in vivo, a phenomenon that can be modeled in vitro. Previously, we identified topoisomerase IIβ (TOP2B) as a novel mediator of RA-resistance in APL cell lines.

Purpose of the study: RA treatment leads to an increase of TOP2B protein levels. However mechanistic data on the causes of this upregulation have been lacking. We therefore sought to investigate the RA-mediated pathways leading to increased TOP2B expression.

Methods: In vitro derived RA-resistant cell lines are useful experimental models for the study of mechanisms of RA-resistance in APL. Our lab has previously isolated an RA-resistant subclone from the parental RA-sensitive cell line NB4, denoted NB4-MR2. Western blot analysis and quantitative real time PCR (qPCR) were used to measure protein and mRNA levels, respectively. Differentiation was assessed by morphological analysis, by immunofluoresence staining of PML nuclear bodies, by expression of the CD11c cell surface myeloid specific antigen, and by nitro-blue-tetrazolium (NBT) reduction.

Results: We speculated that RA may activate protein kinase C delta (PRKCD), leading to increased phosphorylation and stability of TOP2B. The activation of PRKCD by RA correlates with increased TOP2B levels in both NB4 and NB4-MR2 cell lines. Most strikingly, NB4-MR2 cells show substantially increased basal levels of activated PRKCD when compared to NB4. Pharmacological inhibition of PRKCD, targeted knockdown of PRKCD and expression of a dominant negative inhibitory form of PRKCD, all resulted in reduction of TOP2B levels. Additionally, co-treatment with the PRKCD inhibitor, Rottlerin, and RA resulted in the induction of an RA responsive reporter construct, as well as the endogenous RA target genes, CEBPE, CYP26A1 and RIG-I. Furthermore, the co-treatment overcame the differentiation block in the NB4-MR2 RA-resistant cells.

Conclusion: Cumulatively, our data suggest a model whereby inhibition of PRKCD decreases TOP2B protein levels, leading to a loss of TOP2B mediated repressive effects on RA-induced transcription and granulocytic differentiation. Our findings that RA-resistance in APL cells can be overcome by targeting both the PRKCD and RA pathways may provide a basis for the rational design of novel therapies for not only RA-resistant APL, but other more common leukemias that have increased TOP2B expression.

PA018

Title: PLZF-RARa SENSITIZES AML CELLS TO HISTONE DEACETYLASE INHIBITOR INDUCED CELL DEATH

Authors: Petruccelli LA, Pettersson F, Nichol JN, Rice KL, Licht JD, Miller WH Jr.

Affiliation: Dr. Wilson H Miller Jr, McGill University, Department of Experimental Medicine, Jewish General Hospital, Segal Cancer Center/Lady Davis Institute

Keywords: Acute Myeloid Leukemia (AML), Histone Deacetylase Inhibitor (HDACi), DNA damage, cell cycle, Fusion Protein (FP)

Background: HDACi are clinically effective in treating patients with Cutaneous T-cell Lymphoma (CTCL). Despite the success of HDACi in CTCL, their efficacy in other malignancies has been disappointing and mechanisms of HDACi-mediated apoptosis remain ill defined. AMLs often display chromosomal rearrangements resulting in FP like PLZF-RARa. The presence of FP has been associated with a DNA repair deficient phenotype in AML cells. Additionally, HDACi have been shown to inhibit DNA repair and induce DNA damage. Our study sought to determine whether FP expression could sensitize AML cells to HDACi-induced cell death by way of increased DNA damage.

Purpose: The objective of our study is to evaluate mechanisms of increased HDACi-induced cell death in PLZF-RAR□aexpressing cells in order to identify novel pathways that can be targeted to increase the efficacy of HDACi.

Methods: We used the cell line PLZFRAR□3, a sub-line of the myelomonocytic AML cell line U937 that expresses the PLZF-RARα fusion protein when cultured in the absence of tetracycline. We studied the effects of the PLZF-RARa expression on HDACi-induced cell death (DNA fragmentation, caspase activation). DNA damage was assayed using the alkaline comet assay. Additionally, we performed a one-color microarray-based gene expression analysis (Agilent, human whole genome 4x44k microarray). Micoarray data was validated by quantitative real-time PCR (qPCR).

Results: Expression of PLZF-RAR increased sensitivity of AML cells to HDACi-induced cell death as measured by DNA fragmentation beginning at 24h. Activation of upstream caspases, caspase-8 and caspase-9 occurred beginning at 12h. We did not observe an increase in DNA damage when PLZF-RAR expressing cells were treated with HDACi. Preliminary analysis of the microarray found that PLZF-RAR expressing cells treated with HDACi had increased expression of genes implicated in cell cycle regulation.

Conclusions: Our study shows that PLZF-RAR expression significantly sensitizes cells to HDACi-induced cell death. However, our data suggests that DNA damage does not play a significant role in the increased cell death observed. Our preliminary microarray data analysis may suggest the implication of cell cycle regulators as their strong induction never results in any cell cycle arrest.

PO19

Title: B-type cyclins regulate the localization of PAR proteins in the early C. elegans embryo

Authors: Alexia Rabilotta and Jean-Claude Labbé

Affiliation: Department of Pathology and Cell Biology, Université de Montréal, Institute for Research in Immunology and Cancer, Montreal, Quebec, Canada.

Keyword: Asymmetric cell division, Cell cycle, Polarity, C. elegans, Cancer.

Background: Asymmetric cell division is an essential process for the development of multicellular organisms as well as for the division of stem cells and its lost has been proposed to lead to certain forms of leukemia. In order to divide asymmetrically, cells first need to establish an axis of polarity. We are using the C. elegans embryo in order to study the molecular mechanisms underlying the establishment and maintenance of cell polarity. In the zygote, the establishment of an antero-posterior axis of polarity depends on contractility of the actomyosin cortex and on asymmetric localization of PAR proteins in two mutually exclusive cortical groups: the anterior PAR-3/PAR-6/PKC-3 complex and the posterior group with PAR-2 and PAR-1. The absence of any PAR protein leads to a loss of polarity, an abnormal symmetric division and to embryonic lethality.

Purpose of the study: A genome-wide RNAi screen identified two B-type cyclins, cyb-2.1 and cyb-2.2, as suppressors of the lethality caused by the loss of PAR-2. The goal of this project is to characterize the role of these cyclins in cell polarity.

Methods: cyb-2.1 and cyb-2.2 are 97% identical in their nucleotide sequence. To determine whether these genes were redundant, we generated double and triple mutants between par-2(it5ts), cyb-2.1(tm2027) and/or cyb-2.2(tm1969). We used transgenic embryos or immunostaining to follow PAR proteins' localization in cyb-2 mutants and analyzed specific polarity phenotypes by differential interference contrast microscopy.

Results: A mutation in either cyclin suppressed lethality and restored normal cell cycle timing, asymmetric mitotic spindle positioning and anterior PAR protein localization in par-2 mutants. Interestingly, we observed that the localization of PAR-6::GFP was more anterior in cyb-2.2; cyb-2.1 mutants whereas GFP::PAR-2 localization was not affected in cyb-2(RNAi) embryos. This suggests that cyb-2 controls anterior PAR protein localization independently of posterior PAR proteins. This occurs independently of the anchoring of PAR proteins to the cortex, as the cortical vs central cytoplasm ratio of PAR-6 or PAR-3 levels was not affected in cyb-2 mutants. The hyper-polarization of anterior PAR proteins in cyb-2 mutants could involve actomyosin contractility which initiates after the second meiosis. We are currently addressing this by quantifying the timing and the dynamics of contractile polarity during cell cycle cyb-2 mutants.

Conclusion: Our results reveal a novel role for B-type cyclins in C. elegans embryonic polarity, which could underscore a more general functional link between cell cycle regulation and asymmetric cell division. Since these two processes are mis-regulated in leukemia, this work could lead to the identification of novel therapeutics targets to prevent cancer progression.

PO20

Title: E2A and SCL are functional partners that maintain the pool of adult hematopoietic stem cells

Authors: Shanti Rojas-Sutterlin, Julie Lacombe, Sabine Herblot, Stéphane Barakat, André Haman and Trang Hoang

Affiliation: Trang Hoang, IRIC, University of Montreal

Keywords: SCL, E protein, hematopoietic stem cells, quiescence

Background: All blood cells have a limited life-span and are continuously replaced by maturation of progenitor cells, which in turn are derived from hematopoietic stem cells (HSCs). Thus, HSCs ensure the life-long production of blood cells via their self-renewal potential, giving rise upon division to daughter cells with the same unlimited repopulation ability. At the adult stage, the majority of HSCs are quiescent (G0 state). In fact, decreasing the proportion of quiescent HSCs by gene manipulation impaired their long-term self-renewal potential, indicating that HSC quiescence is an important determinant of self-renewal capacity. We have recently shown that SCL (stem cell leukemia), a bHLH transcription factor, controls the G0-G1 transition in HSCs and their long-term self-renewal potential at the adult stage. SCL is part of a multifactorial complex composed among others of E proteins, as E2A and HEB.

Purpose of the study: The goal of this study is to investigate the importance of E2A and HEB in HSC cell cycle control and long-term competence.

Methods: We have used two strategies to measure the proportion of quiescent cells in the HSC pool. First, we assessed the cell cycle distribution of freshly isolated bone marrow (BM) cells by staining their DNA and RNA content with Hoechst and Pyronin Y or Ki67, respectively. Flow cytometry analysis allowed us to discriminate the G0, G1 and S/G2/M states of the cell cycle in HSC enriched population. Second, we demonstrated a higher cycling status of HSCs by in vivo functional study. 5-fluorouracil (5-FU) is a drug that specifically kills actively proliferating cells, therefore not affecting quiescent HSCs. Consequently, mice with increased proportion of cycling HSCs will be more sensitive to 5-FU treatments. Thus, we monitored the survival outcome of mice of various genotypes following two 5-FU treatments. Finally, we monitored HSC long-term self-renewal capacity by transplantation assays into irradiated hosts.

Results: As Scl, qPCR analysis showed that E2a and Heb are highly expressed in BM population enriched for HSCs with long-term self-renewal potential, suggesting a role of those E proteins in HSCs. Supporting the qPCR analysis, a knock-down of Scl, E2a or Heb affects the balance between quiescent and cycling HSC in vivo. First, the proportion of HSCs in G0 state is reduced to differing degrees compared to wild-type (wt) HSCs when Scl, E2a or Heb are decreased either by loss of one allele or by the shRNA technology. Second, the survival outcome of heterozygous mice for Scl, E2a or Heb following 5-FU treatments indicates a greater sensitivity to cytotoxic injury, suggesting that a larger proportion of HSCs are cycling in these mice. In addition, these HSCs have decreased long-term reconstitution potential in transplantations assays. Finally, genetic approaches indicate that SCL and E2A are functional partners in HSCs.

Conclusion: Our results indicate that as SCL, E2A and HEB are implicated in HSC quiescence control and that SCL together with E2A regulate HSC long-term competence.

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PO21

Title: Regulation of IkB ubiquitination and activity by β-adrenergic ligands: the role of βarrestin2.

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Keywords: IkB, NFkB, β-adrenergic receptors, βarrestin, ubiquitination, leukemia

Background: Constitutive activation of transcription factor, NFkB, has been linked to the development of various forms of leukemia. Activation of NFkB and nuclear translocation is controlled by the targeted phosphorylation and subsequent degradation of IkB and associated with increased transcription of genes encoding antiapoptotic proteins, proinflammatory cytokines, and cell-adhesion molecules. Therefore, NFkB and its regulators have been seen as major targets for anticancer drug development. Recently, a growing number of reports suggest that, in addition to its tumour promoter action, NFkB can also act as a repressor of tumour growth. Moreover, NFkB activity is modulated by tumour suppressor p53 and the scaffolding protein βarrestin (βarr). It has been shown that βarr promotes stabilization of IkB and controls the ubiquitination of p53 by the E3-ligase, MdM2: βarr could thus play a central role in the coordinated regulation of NFkB and p53. Interestingly, βarr interactions with IkB and MDM2 can be affected by G protein-coupled receptor (GPCR), such as the β2-adrenergic receptor (β2AR). The fact that βARs are expressed in almost all lymphoid cells suggests that they may represent pharmacological targets to manipulate the βarr-mediated control of NFkB and p53, and thus, control the balance between their tumour promoter and tumour suppressor activity.

Purpose of the study: This study was designed to explore the coordinated regulation of NFkB and p53 in response to βAR activation. For this purpose, we investigated the physical and functional interactions between NFkB, IkB, βAR, βarr and p53.

Methods: To directly study the interactions between the various partners, we used bioluminescence resonance energy transfer (BRET)-based assays that allow monitoring the dynamics of protein-protein interactions in living cells. The assay is based on the fact that the energy derived from a luciferase reaction can be used to excite the fluorescent proteins, if the latter is in close proximity to the luciferase enzyme. Combination between BRET and another fluorescence resonance energy transfer (FRET) method permits to simultaneously visualize the dynamics of association between the partners not only in a pair-wise fashion but also as part of complexes involving 3 or 4 partners simultaneously. NFkB activity was measured using a gene-reporter assay system. To verify the role of each of the proteins involved in the signalling pathways, we used complementary strategies such as western blot analyses, site directed mutagenesis and genetic down-regulation using siRNA in cell culture systems.

Results and conclusions: We demonstrated that βarr interacts directly with IkB, and that overexpression of βarr leads to a marked inhibition of NFkB activity. Stimulation of either β1AR or β2AR by the β-adrenergic agonist, isoproterenol, significantly enhanced the βarr-IkB interaction, decreased the ubiquitination of IkB and inhibited NFkB activity. In addition to the enhanced βarr-IkB interaction, activation by various β-adrenergic ligands such as isoproterenol, epinephrine, norepinephrine and carvedilol promoted the recruitment of both βarr and IkB to the β1AR and/or β2AR, leading to the formation of a ternary complex between βarr, IkB and the receptors at the plasma membrane. Consequently, βAR activation can modulate NFkB activity and expression of NFkB target genes in a βarr-dependent manner by preventing the ubiquitination of IkB and stabilizing the NFkB-IkB complex in an inactive form at the plasma cortex. When considering p53, we confirmed that βarr interacts directly with MDM2, the E3 ubiquitin ligase of p53, and found that this interaction was increased by βAR activation. Whether MDM2 is present in the βarr-IkB-NFkB-receptor complex or associated with a different pool of βarr remains to be established. No direct interaction was observed between βarr and p53 but, intriguingly, we observed an association between p53 and IkB. Whether this interaction is regulated by βAR activation, βarr recruitment or the ubiquitination state of the partners is currently under investigation. Taken together, our data indicate that activation of GPCRs, such as the βARs, can regulate the activity of NFkB, opening the way for the development of pharmacological interventions targeting GPCRs for tumour suppression action. Our data also points at βarr as an important integrator between GPCR, p53 and NFkB signalling pathways that could be exploited for therapeutic development.

P022 *(not present)*

Title : The oncoprotein LMO2 regulates DNA replication in hematopoietic cells

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Keywords : LMO2, DNA replication, T-ALL, hematopoiesis

Background information: 70% of chromosomal rearrangements in acute leukemia involve oncogenes that code for transcription factors, but the molecular mechanism through which they transform hematopoietic cells remain to be clarified. This is exemplified by the oncogenic transcription factor LMO2, activated in childhood acute lymphoblastic T cell leukemias (T-ALL). LMO2 is a very small protein that lack direct DNA-binding ability. In addition to its known role in transcription, LMO2 could mediate the assembly of non-transcriptional protein complexes.

Purpose of the study: In this study, we investigated whether LMO2 could be part of new protein complexes. This information could reveal new mechanisms of oncogenicity by LMO2 and could serve as a paradigm for other types of leukemias and/or cancers.

Methods: We identified new LMO2-interacting proteins by yeast two hybrid, using a hematopoietic progenitor cDNA library. The interactions have been confirmed by immunoprecipitation in hematopoietic cells. We investigated the involvement of LMO2 in the DNA replication process by the use of shRNAs against LMO2 in an hematopoietic cell line, and by LMO2-overexpressing bone marrow transplantation in mice.

Results: We provide evidence that ectopic LMO2 expression specifically influences cell cycle in thymocyte progenitors, which are targets of cell transformation in T-ALL, but not marrow-derived progenitor cells. LMO2 is recruited to several known origins of replication in hematopoietic cells by direct interaction with three essential components of the pre-initiation (pre-IC) replicative complex: polymerase delta, primase 1 and MCM6. This interaction with the pre-IC occurs on DNA and is distinct from LMO2 interaction with the SCL-transcription complex which can occur in the absence of DNA. In addition, LMO2 levels are highest in G1 and at the G1-S transition, and decrease in late S and G2M. Accordingly, LMO2 co-localizes with MCM5/6 during the G1-S transition. Finally, disrupting LMO2 levels delays S phase progression in hematopoietic cells.

Conclusion: We surmise that LMO2 controls G1-S transition by favouring the assembly of pre-initiation complexes on DNA. We show that LMO2 associate with replication proteins MCM2-7, DNA primase and DNA polymerase delta, and that LMO2 influences cell cycle progression in hematopoietic cells. Our results support a new role for LMO2 in the DNA replication process, which may be a new mechanism of cell transformation by LMO2 that could apply to other oncogenes. A better understanting of the molecular role of LMO2 in leukemogenesis will pave the way to the development of targeted inhibitors that may specifically antagonize the growth of cancer cells.

PO23

Title: Modeling targeting of the RNA binding protein hnRNPA1 in B-cell lymphomagenesis

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Keywords: shRNA, B-cell lymphoma, translation, hnRNPA1, transgenic animals

Background: The Eu-myc mouse model is a model of lymphomagenesis, a transgenic mimic of the translocated myc genes found in lymphoid tumors. The c-myc gene encodes a transcription factor that is a key regulator of proliferation and its expression is dysregulated in many cancers, including Burkitt's lymphoma and multiple myeloma (MM). Myc overexpression is driven by a special mechanism involving internal recruitment of ribosomes, called IRES-mediated translation (1). The RNA binding protein, hnRNP A1 is a well studied ubiquitously expressed protein, that has been implicated in translational control of c-myc (and cyclin D1) IRES activity (2). Knockdown of hnRNPA1 renders quiescent Akt positive cells sensitive to rapamycin-induced G1 arrest and support a role for hnRNPA1 in mediating rapamycin-induced alterations of c-myc IRES activity in an Akt-dependent manner (2).

Purpose of the study: The purpose of this study is to model knockdown of hnRNPA1 using inducible and reversible RNA Interference on Eu-myc driven lymphomagenesis.

Methods: We are using a reversible and inducble gene targeting system to engineer a mouse model in which hnRNP A1 expression can be knocked down. This involves site-directed integration in an ES cell line using a targeting cassette harbouring a microRNA-based shRNA. Once established, mice harbouring shRNAs to hnRNP A1 will be crossed to Eu-myc mice. Expression of the shRNAs can be induced in these animals by feeding them Doxycyline and the requirements for hnRNP A1 assessed for tumor establishement and maintenance.

Results: To model knockodown of hnRNP A1 in myc-driven B cell lymphomagenesis, we have designed and tested shRNAs to hnRNPA1. shRNAs showing knockdown efficiencies of >80% were chosen and cloned into a targeting vector for homologous recombination into ES cell lines. We have also shown that knockdown of hnRNP A1 is not lethal to cells. These were used to establish stable ES cell lines showing inducible hnRNP A1 knockdown. Currently transgenic mice are being developed with these ES lines.

Conclusions: A unique gene targeting system has been established and shRNAs to hnRNPA1 screened . Using this system, we will be able to model knockdown of hnRNPA1 in myc-driven B cell lymphomagenesis (and other cancers) to assess the requirements for hnRNP A1 on tumor initiation and maintenance.

PO24

Title: Modeling T-cell acute lymphoblastic leukemia induced by the SCL and LMO1 oncogenes

Authors: Mathieu Tremblay, Cédric S. Tremblay, Sabine Herblot, Peter D. Aplan, Josée Hébert, Claude Perreault and Trang Hoang

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Keywords: T cell acute lymphoblastic leukemia, SCL, LMO1, Notch1, pre-TCR

Background: Deciphering molecular events required for full transformation of normal cells into cancer cells remains a challenge. In T-cell acute lymphoblastic leukemia (T-ALL), the genes encoding the TAL1/SCL and LMO1/2 transcription factors are recurring targets of chromosomal translocations whereas NOTCH1 is activated in more than 50% of patients.

Purpose of the study: The goal of the study is to adress the significance of important T cell signaling pathways (pre-TCR and NOTCH1) in cells transformation by the SCL and LMO1 oncogenes.

Methods: Using molecular, cellular and genetic approaches in a mouse model of T-ALL, we adressed the nature of the cells that are susceptible to transformation during the preleukemic stage, and characterized the population of leukemic stem cells.

Results: Here we show that at the preleukemic stage, the SCL and LMO1 oncogenes collaborate to expand primitive thymocyte progenitors and inhibit later stages of differentiation. Together with pre-TCR signaling, these oncogenes provide a favorable context for the acquisition of activating Notch1 mutations and the emergence of self-renewing leukemia-initiating cells in T-ALL. All tumor cells harness identical and specific Notch1 mutations and Tcr clonal signature, indicative of clonal dominance and concurring with the observation that Notch1 gain of function confers a selective advantage to SCL-LMO1 transgenic thymocytes. Accordingly, a hyperactive Notch1 allele accelerates leukemia onset induced by SCL-LMO1 and by-passes the requirement for pre-TCR signaling. Finally, the time to leukemia induced by the three transgenes corresponds to the time required for clonal expansion from a single leukemic stem cell.

Conclusions: Our results suggest that SCL, LMO1, and Notch1 gain of function together with an active pre-TCR might represent the minimum set of complementing events for the transformation of susceptible thymocytes.

PO25

Title: Defining the molecular mechanisms involved in normal karyotype acute myeloid leukemias

Author: Dr. Brian Wilhelm

Affiliation: Dr Guy Sauvageau, Department of Medicine, Institute for Research in Immunology and Cancer

Keywords: AML-NK, Next-gen sequencing, DNA microarrays, BCLQ

Background information: Every year in Canada, thousands of new cases of Acute Myeloid Leukemia (AML) will be diagnosed and of these, nearly half will appear normal under the microscope. Although a small number of genes have been found to be recurrently altered in AML-NK, these changes are not essential for development of AML and do not allow patients to be effectively classified into treatment groups. These observations strongly suggest that there are other mutations that are involved in the development of AML-NK.

Purpose of the study: The purpose of this project is to identify novel mutations which are present in AML-NK in order to allow better patient stratification in the short term and to enable the development of rational and effective treatment in the longer term.

Methods: To study genomic changes specifically associated with AML-NK, highly characterized AML patient bone marrow samples have been obtained from the Quebec Leukemia Cell Bank, through the assistance of Dr. Josée Hébert. A number of these were characterized by high resolution aCGH using Nimblegen microarrays, and although recurrent deletions could be detected and in some instances validated, the majority of changes in these tumors cannot be detected by microarray-based approaches. Given the possibility of using next generation sequencing to characterize AML tumors, a pilot project using two mouse leukemias was devised to validate the approach of performing RNA sequencing (RNA-seq) on tumors. Having confirmed that this is a viable approach we have now begun performing RNA-seq and directed exome sequencing on several AML-NK patients.

Results: The initial results of the aCGH work did confirm the deletion of a large portion of the HoxA cluster in one patient, however other deletions could not be validated using robust cytogenetic methods. Given very recent data published from AML-NK tumor genomes suggesting that the large majority of mutations at single base pair changes, a pilot project to use RNA-seq on leukemia samples was started. This project generated a remarkably clear picture of the gene expression pattern of two closely related leukemias and identified two single nucleotide changes which may be responsible for the significant differences in leukemic stem cell frequencies. The RNA-seq data revealed extensive structural differences in the transcriptomes of the leukemias as well as wide spread transcription of novel elements, indicating that even closely related leukemias can have quite divergent transcriptomes. Human AML-NK samples are now being sequenced along with the patient matched normal DNA from all coding exons in order to identify tumor specific variations.

Conclusion: I have demonstrated that cutting edge technology such as high resolution aCGH and next generation sequencing can be applied to understand the molecular mechanisms behind AML. Using these high throughput approaches on tumor samples from five AML patients will provide an enormous amount of information regarding novel genes involved in the development of the disease as well as to identify potential genetics targets for patient stratification and future drug development.

PO26 *(not present)*

Title: Role of BAP1 Transcriptional Complex in Leukemia

Authors: Helen Yu, Nazar Masthalir, Salima Daou, Ian Hammond Martel, Amélie Giguère, Josée Hébert, Elliot Drobetsky et El Bachir Affar.

Affiliation: Centre de recherche de l'Hôpital Maisonneuve-Rosemont, Hôpital Maisonneuve-Rosemont, Université de Montréal.

Keywords: Leukemia; transcription; deubiquitinase; cell cycle

Background: Genetic abnormalities of transcription regulators are major determinants in leukemia pathogenesis. Our laboratory is intensively investigating the role of ubiquitin signaling in chromatin-associated processes and carcinogenesis. Ubiquitination is a post-translational modification, which can be reversed by deubiquitinases (DUBs). It is clear now that DUBs are important enzymes with central roles in carcinogenesis.

The DUB BAP1 is an ubiquitously expressed protein mutated in a subset of cancers, although the function of this enzyme remains poorly understood. In the course of investigating the biological function of BAP1, we found that this protein is required for cell cycle progression. To understand how BAP1 regulates cell cycle, we purified its interacting proteins. We found that the transcription factor Yin Yang 1 (YY1) and the polycomb group protein ASXH2 are tightly complexed with BAP1. Both BAP1-associated proteins are involved in acute myeloid leukemia (AML).

Purpose of the study: Our results allowed us to hypothesize that BAP1 regulates the expression of genes involved in cell cycle progression. This function is deregulated during leukemiogenesis. The objectives of this project are:

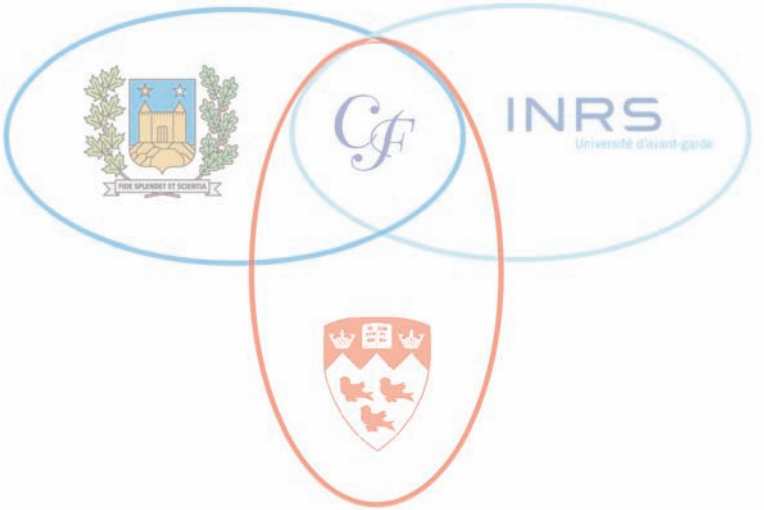
1) To determine the expression profile of BAP1 in cells derived from leukemia patients.
2) To characterize the exact role of BAP1 in cell cycle progression.
3) To define the role of the BAP1 complexes in transcription regulation.

Methods:
1) In collaboration with Dr. Hébert at HMR, we have determined the expression levels of BAP1 in cells derived from leukemia patients (Quebec leukemia cell bank) by RT-PCR and Western Blot.
2) We have characterized the cell cycle profile of cells depleted from BAP1 by RNAi. This analysis was conducted using propidium iodide staining and flow cytometry.
3) BAP1 complexes contain transcription factors suggesting a role of BAP1 in regulating gene expression. Thus, we performed transcription reporter assays to study the transcriptional activity of BAP1. Next, we conducted microarray experiments to identify BAP1 target genes and used chromatin immunoprecipitation for validation.

Results: On 32 leukemia patient cells, 2 have BAP1 downregulated. Interestingly, these two patients have a complex karyotype, a characteristic feature of genomic instability. Moreover, we found that BAP1-depleted cells have a defect in S phase of the cell cycle. Next, global gene expression analysis revealed that BAP1 regulates the expression of critical genes of the cell cycle (skp2, p107, cdc6, cdt1, cdc25a and the mcms). We further demonstrated that BAP1 activates transcription in a DUB activity-dependant manner. Finally, we showed that BAP1 is recruited by YY1 on the promoters of specific genes in vivo.

Conclusion: Our study showed that BAP1 is an important regulator of cell proliferation by controlling the expression of critical genes of the cell cycle. Further studies need to be conducted to understand the exact mechanism of action of BAP1 and to determine how deregulation of its function contributes to leukemia development. Thus, understanding the molecular basis of the disease will help us to provide better therapeutic treatments.

Notes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