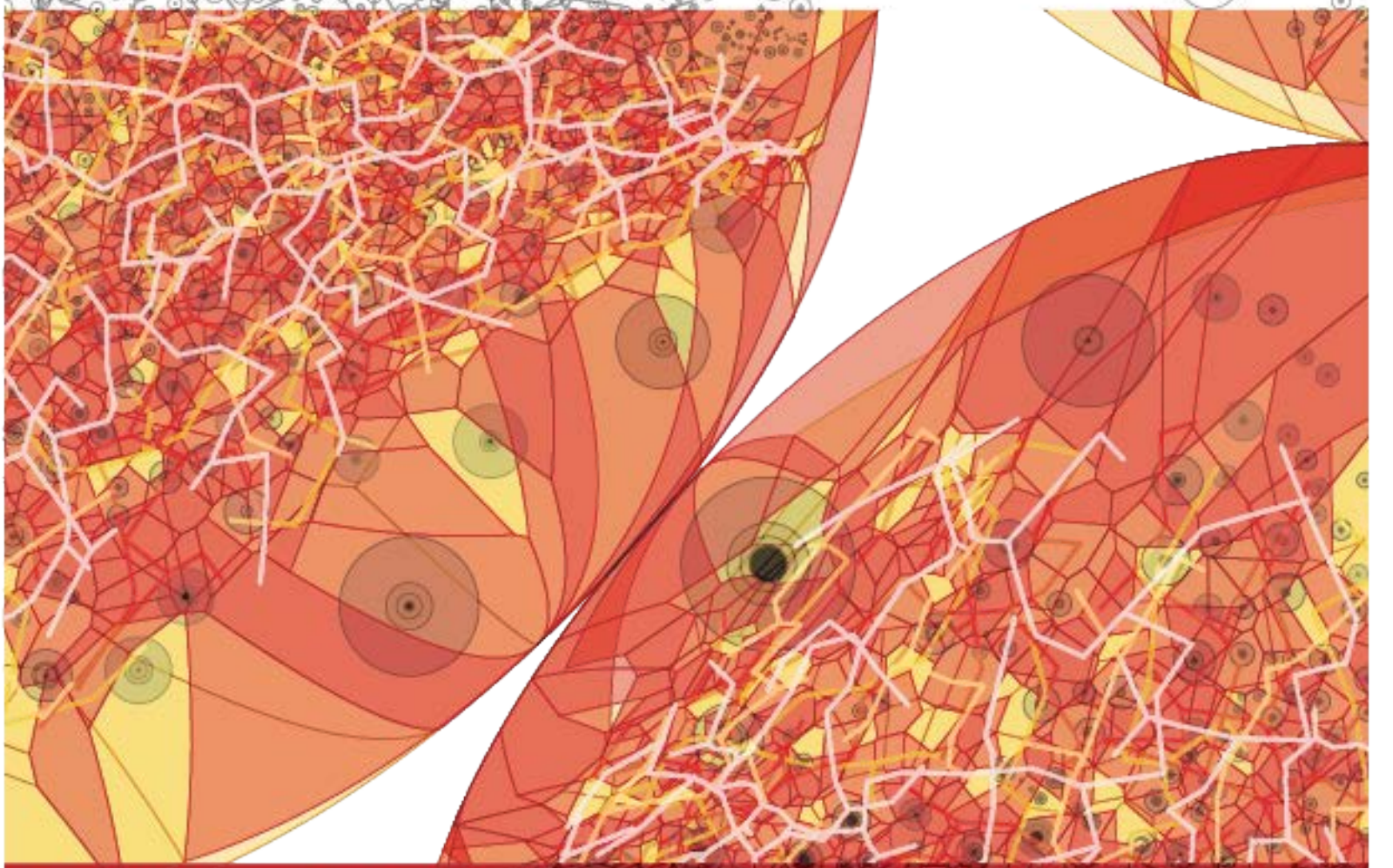
CEEHRC NETWORK
Canadian Epigenetics, Environment and Health Research Consortium Network

THE 4TH CANADIAN CONFERENCE ON

EPIGENETICS

Mechanisms of Disease



26–29 November 2017 · Westin Resort & Spa · Aava Whistler Hotel · Whistler, BC

EPIGENETICS CANADA

GenomeCanada

CIHR IRSC

TOURISM WHISTLER

Welcome!

Dear Colleagues,

It is a pleasure to welcome you to the Whistler Westin Resort & Spa for the 4th Canadian Conference on Epigenetics: “Mechanisms of Disease”.

It has been an exciting year for epigenetic research in Canada and beyond. This year our community continued to contribute to a growing body of evidence providing mechanistic understanding and support to the existence of transgenerational epigenetic inheritance in animals. We have seen coalescence towards enhancer state disruption as a common mode of action in response to genetic and metabolic inactivation of epigenetic modifiers in disease. New therapeutics targeting pathogenic epigenetic states continue to show promise in clinical trials with many more in development. The International Human Epigenome Consortium released a second round of human reference epigenomes and new methodologies have allowed for the measurement of epigenetic features with ever increasing cellular and molecular resolution while also reaching into new dimensions of epigenetic control. Intriguing correlations between epigenetic signatures and environmental influences continue to be reported and mechanistic understanding of the phenomena underpinning environmentally influenced epigenetic state changes in model systems has emerged.

This year also saw the launch of phase II of the Canadian Epigenetics and Environment Health Research Consortium (CEEHRC) and the development of the Canadian Epigenetics National Centre of Excellence/ Centre d’Excellence National Canadien en Épigénétique. Phase II of CEEHRC includes continued funding to the CEEHRC mapping Centres with dedicated capacity for epigenomic mapping made available to the Canadian epigenetic research community. During the symposium, we will provide details on this capacity and consult with CEEHRC membership to develop a mechanism for access to this resource.

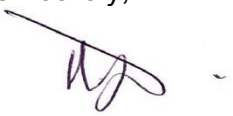
This symposium is intended to bring together Canadian epigenetics researchers, along with key international leaders in the field, to engage in cross-disciplinary dialogue on recent advancements in the field of epigenetics with a focus on the impact of epigenetics on human disease. The scientific program includes distinguished invited speakers in the fields of epigenetics and epigenomics covering topics including basic chromatin biology, epigenetic profiling, epigenetic markers, and new developments and advancements in epigenomics research. These topics will be covered over 7 sessions that include plenary lectures and short oral presentations, a data visualization workshop, and 2 poster sessions.

We especially encourage interaction and networking between young Scientists and Principal Investigators, and hope to foster an atmosphere that encourages scientific collaboration and discussions about science and careers in the field of epigenetics and epigenomics.

This year's meeting is coordinated by the CEEHRC Network, in an ongoing collaboration with the organizers of the successful "Epigenetics, EH!" conferences. The aim of the Network is to connect Canadian epigenetics researchers and expand their reach to the broader health research community in Canada and beyond. We also facilitate access to epigenomics data; produce and curate epigenetic and epigenomics tools, software and protocols; and curate Canadian jobs, training opportunities and events listings. More information is available at www.epigenomes.ca. We welcome your feedback on what you'd like to see from future meetings, and from the Network in general.

On behalf of the Organizing Committee, we wish you a warm welcome to Whistler.

Sincerely,



Martin Hirst, PhD
Head, Epigenomics
BC Cancer Genome Sciences Centre
Director, Canadian Epigenetics, Environment and Health Research Consortium Network
Associate Director, Michael Smith Laboratories
Associate Professor, Department of Microbiology & Immunology
University of British Columbia

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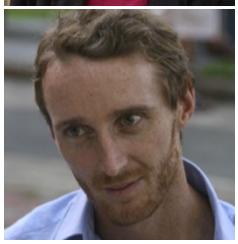
Organizing Committee

Dr. Cheryl Arrowsmith
University of Toronto

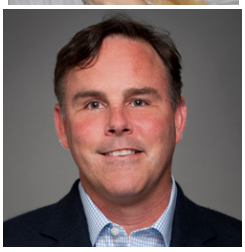
Dr. Nathalie Bérubé
Western University



Dr. Guillaume Bourque
McGill University

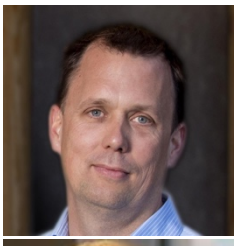
Dr. Carolyn Brown
University of British Columbia

Dr. Jim Davie
University of Manitoba



Dr. Jeffrey Dilworth
University of Ottawa

Dr. Steven Jones
BC Cancer



Dr. Martin Hirst
University of British Columbia

Dr. Tony Kwan
McGill University

Dr. Tomi Pastinen
McGill University

Ms. Sunshine Purificacion
BC Cancer

Speakers

Keynote Speaker

Anne Ferguson-Smith
Head of Department of Genetics, University of Cambridge

Prof. Anne Ferguson Smith, Ph.D. works as Professor of Developmental Genetics at the University of Cambridge and contributes to a range of national and international review panels and initiatives at the interface of genomic and epigenomics research. Prof. Smith has established an international reputation for her work integrating the epigenetic control of genome function with developmental and physiological processes relevant to health and disease. She has more than two decades of experience in the exploration of links between gene regulation, large and small non-coding RNAs and epigenetic modifications, and the consequences of perturbations in these interactions for pre and postnatal well-being.

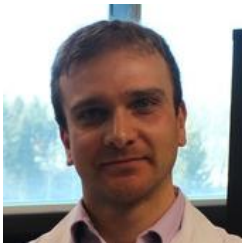
Invited Speakers

Jordana Bell
Head of Epigenomics Research Group, King's College London

Dr. Bell is a Senior Lecturer in the Department of Twin Research and Genetic Epidemiology at King's College London. Her group uses computational methods to study the genetic basis of complex traits in human populations, including intermediate and end-point phenotypes. Their primary research focus is on understanding the causes and consequences of epigenetic variation in humans. Many projects in her lab are multidisciplinary and combine the analysis of large-scale genomic datasets with statistical genetic and epigenetic tools.

Joe Costello
Professor in Residence of Neurological Surgery, UCSF

Dr. Costello is a Professor of Neurosurgery at UCSF and holds the Karen Osney Brownstein Endowed Chair in Neuro-oncology. He is the Director of the NIH-supported Training Program in Translational Brain Tumor Research at UCSF, and recently served as the Director of the UCSF-based NIH Roadmap Epigenome Mapping Center. He is an Associate Member of Canada's Michael Smith Genome Sciences Centre.



Marco Gallo
Assistant Professor, University of Calgary

Dr. Marco Gallo received his PhD in Medical Genetics from The University of British Columbia (2005-10), and pursued postdoctoral studies at The Hospital for Sick Children, Toronto, ON (2010-15) under the supervision of Dr Peter Dirks. Since 2015, he has been Assistant Professor at the University of Calgary, Calgary, AB where his main interest is to understand how the epigenome defines self-renewal hierarchies in brain tumors. His ultimate goal is to identify new and effective ways to target cancer stem cells in brain malignancies.

Benjamin Garcia
Presidential Prof. of Biochemistry and Biophysics, University of Pennsylvania

Dr. Benjamin A. Garcia received his PhD in Chemistry from the University of Virginia under the supervision of Prof. Donald Hunt (2005), and then was an NIH NRSA Postdoctoral Fellow at the University of Illinois with Prof. Neil Kelleher (2005-2008). In 2008, Dr. Garcia was appointed as an Assistant Professor at Princeton University until his recruitment as the Presidential Associate Professor of Biochemistry and Biophysics, and Faculty Director of the Quantitative Proteomics Resource Core at the University of Pennsylvania School of Medicine in 2012, and was promoted to full Professor in 2016.

Anne-Claude Gingras

Senior Investigator, Lunenfield-Tanenbaum Research Institute

Dr. Gingras completed PhD studies in biochemistry at McGill University in Montreal, and obtained postdoctoral training at the Institute for Systems Biology in Seattle, before moving to Toronto and joining the Lunenfeld in 2005. She also began teaching at the University of Toronto in 2006 in the Department of Molecular Genetics.

Tatiana Kutateladze

Professor, Department of Pharmacology, University of Colorado

Dr. Kutateladze is a Professor of Pharmacology at the University of Colorado School of Medicine. Research interests include studying epigenetic mechanisms, posttranslational histone modifications and the role of epigenetic misregulations in human disease, cancer in particular. Her laboratory has substantially contributed to deciphering the epigenetic language through characterizing structures and functions of chromatin-binding and chromatin-remodeling complexes. Her laboratory applies the latest breakthroughs in high field NMR spectroscopy and X-ray crystallography to unveil the atomic-resolution structures and mechanistic aspects of chromatin-interacting proteins involved in transcriptional regulation and DNA damage repair and implicated in cancer.

Mathieu Lupien

Associate Professor, Dept of Medical Biophysics, University of Toronto.

Dr. Mathieu Lupien is a senior scientist at the Princess Margaret Cancer Centre, associate professor in the Department of Medical Biophysics at the University of Toronto, and cross-appointed with the Ontario Institute for Cancer Research. He earned his PhD at McGill University, followed by post-doctoral training in medical oncology at the Dana-Farber Cancer Institute, Harvard Medical School as an Era of Hope fellow. Dr. Lupien completed his post-doctoral training in 2008 and was recruited as a faculty member at the Dartmouth Medical School, where he became Director of the Quantitative Epigenomics Laboratory before moving to Princess Margaret Cancer Centre in 2012.

Jacek Majewski

Associate Professor, Department of Human Genetics, McGill University

Dr. Majewski received his PhD in Evolutionary Biology from Wesleyan University, followed by post-doctoral training at Rockefeller University. He is Associate Professor, Department of Human Genetics, Faculty of Medicine at McGill University and holds the Canada Research Chair in Statistical Genetics. Pr. Majewski's research is based on genomics and bioinformatics analysis of high throughput data. The recent revolution in massively parallel DNA sequencing has opened new venues into numerous biomedical problems.

Shyam Prabhakar

Associate Director, Integrated Genomics, Genome Institute of Singapore

Dr. Prabhakar obtained his PhD in Applied Physics from Stanford University and received the American Physical Society thesis award for Beam Physics in 2001. Following a PDF at the Lawrence Berkeley National Laboratory, he started his own research group at the Genome Institute of Singapore (GIS). His lab uses single-cell RNA-seq, cohort-scale ChIP-seq and other high-throughput assays, together with algorithm development, to uncover markers and molecular mechanisms of human diseases. Major achievements include the first single-cell transcriptomic analysis of colorectal cancer, the first large-scale screen for disease-causing genetic variants that affect histone acetylation, the first histone acetylome-wide association study of a psychiatric disorder and the first unified signal-processing method for peak detection in NGS data. Dr. Prabhakar is also GIS lead for the centre for Big Data and Integrative Genomics (c-BIG).

Wendy Robinson
Professor, Department of Medical Genetics, University of British Columbia

Dr. Robinson completed her Ph.D. in Population Genetics at UC Berkeley and a postdoctoral fellowship in Zurich, Switzerland. She believes in approaching problems from multiple angles and questioning everything you read. She is the proud mother of two great boys and is very appreciative of all the students/staff who do all the work!

Jacquetta Trasler
Prof. Pediatrics / Human Genetics / Pharmacology & Therapeutics, McGill

Jacquetta Trasler is a James McGill Professor in Pediatrics, Human Genetics and Pharmacology & Therapeutics at McGill University and Senior Scientist in the Child Health and Human Development Program at the Research Institute of the McGill University Health Centre. Dr. Trasler received her MD and PhD degrees from McGill University followed by postdoctoral training in reproductive molecular biology at Tufts University in Boston. Her research interests focus on epigenetics and the molecular and developmental regulation of gene expression in the germline and early embryo. Dr. Trasler's ongoing studies include effects of drugs, diet (folate) and assisted reproductive technologies on the epigenome of germ cells and embryos and the implications for intergenerational passage of epigenetic defects.

Rosanna Weksberg
Professor, Molecular and Medical Genetics, University of Toronto

Rosanna Weksberg, MD, PhD, is a Professor of Pediatrics and Molecular Genetics at The Hospital for Sick Children and the University of Toronto. She was Head, Division of Clinical and Metabolic Genetics, The Hospital for Sick Children for 11 years (1998-2008). She has over 100 publications in the areas of epigenetics, imprinting and growth control. She has worked on the overgrowth and cancer predisposition syndrome-Beckwith-Wiedemann syndrome and related conditions since 1995. Over the last 10 years, her research has also focused on elucidating the biological basis and environmental contributors to neurodevelopmental disorders such as early onset psychosis and autism.

Workshop Speaker

Martin Krzywinski
Staff Scientist, Bioinformatics, BC Cancer - Genome Sciences Centre

As scientists, it is our responsibility not only to present our work clearly, but to connect broadly by sparking imagination and enthusiasm for inquiry and understanding. To do this, science communication must engage both cognitively and emotionally. I try to make people think and feel good by creating visualization tools and information graphics that combine analytical clarity with an artistic dimension. Real science should look just like in movies. My introduction to genomics was as a system administrator at Canada's Michael Smith Genome Sciences Center, where in 1999 I built its first computing infrastructure, and contributed to IT security, optimized keyboard layouts and created database visualizations. During my work on cancer genomes, I created Circos, now a community standard for displaying information in this field. More recently, I introduced a method for rationally visualizing networks using hive plots.

Selected Speakers

Jean-François Couture, PhD

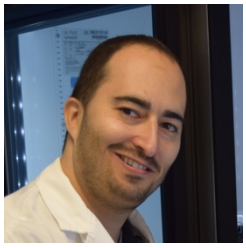
Professor, Department of Biochemistry, Microbiology & Immunology, University of Ottawa

Dr. Jean- François Couture is an Associate Professor in the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa, and holds the Canada Research Chair in Structural Biology and Epigenetics. His lab is working to understand the role of the mixed-lineage leukemia (MLL) gene, and why MLL leukemia is so difficult to treat.

Michael Hendzel, PhD

Professor, Department of Cell Biology, University of Alberta

Michael Hendzel completed a Ph.D. studying the dynamics of histone posttranslational modifications in 1993 from University of Manitoba under the supervision of Dr. James R. Davie. He next completed a postdoctoral fellowship in the cell biology of the nucleus under the supervision of Dr. David Bazett-Jones at the University of Calgary. He has been a member of the Department of Oncology at the University of Alberta since 1999, where he is currently a full professor. His laboratory currently focuses on the development of electron microscopy techniques for the study of the cell nucleus, the role of epigenetic processes in the regulation of DNA double-strand break repair, and the structure and dynamics of interphase chromatin.



Eric Campos, PhD

Assistant Professor, Department of Molecular Genetics, University of Toronto

Eric Campos is a new investigator at the Hospital for Sick Children, and Assistant Professor at the University of Toronto. He obtained his PhD from the University of British Columbia, and continued his postdoctoral training under the supervision of Danny Reinberg at the New York University School of Medicine. His research program focuses on histones, histone chaperones, and the transmission of epigenetic information throughout cell division.

Maxwell Libbrecht, PhD

Assistant Professor, School of Computing Science, Simon Fraser University

Maxwell Libbrecht is an Assistant Professor in Computing Science at Simon Fraser University. He received his PhD in 2016 from the Computer Science and Engineering department at University of Washington, advised by Bill Noble and Jeff Bilmes. He received his undergraduate degree in Computer Science from Stanford University, where he did research with Serafim Batzoglou. His research focuses on developing machine learning methods applied to high-throughput genomics data sets. He was the first author of a paper named one of ISCB's Top 10 Regulatory and Systems Genomics papers of 2015.

Serge McGraw, PhD

Assistant Professor, Department Obstétrique-Gynécologie, Université de Montréal

Serge McGraw is an Assistant professor-researcher at the Centre de recherche du CHU Sainte-Justine et Université de Montréal, and is appointed to the Department Obstétrique-Gynécologie, Faculté de médecine. His principal research interests are focused on the adverse developmental outcomes caused by epigenetic instabilities arising from an interruption in DNA methylation maintenance during early embryogenesis. His laboratory aims at understanding how, during embryo development, perturbations in the embryo epigenetic program via harmful environmental insults alter specific brain-related programs and lead to adverse outcomes in children.

Jennifer Mitchell, PhD
Associate Professor, Department of Cell & Systems Biology, University of Toronto

Dr Mitchell is an associate professor at the University of Toronto in the Department of Cell and Systems Biology where she uses CRISPR/Cas9 genome engineering to study the mechanisms through which transcriptional regulatory elements activate gene expression in stem cells. Dr Mitchell received her PhD from the University of Toronto and conducted her research on transcription factors regulating gene expression in the uterus during pregnancy at The Lunenfeld-Tanenbaum Research Institute with Stephen Lye. Dr Mitchell then pursued Postdoctoral training at the Babraham Institute in Cambridge UK where she worked with Peter Fraser to investigate how genome organization regulates transcription in erythroid cells. Dr Mitchell started her own research group at the University of Toronto in 2009 where she investigates transcriptional mechanisms regulating pluripotency and lineage commitment during development.

J. Richard Pilsner, PhD
Assistant Professor, Division of Environmental Health Sciences, UMass Amherst

It has been long recognized that maternal environmental exposures during pregnancy influence the health and development of offspring. However, little attention has been given to the contribution of paternal environmental exposures to offspring health and development, and more importantly, the pathway(s) by which this paternal transmission may occur. To answer these questions, our research in the lab addresses the interface of environmental toxicology, epidemiology, and reproductive health with an emphasis on epigenetic mechanisms, and specifically to understand the role of sperm epigenetics as a pathway linking paternal environmental exposures to reproductive and offspring health. We are currently investigating sperm epigenetics via two arms of research: our population-based cohort, the Sperm Environmental Epigenetics and Development Study (SEEDS) and in mice models, both supported by the NIH. Dr. Pilsner is an Assistant Professor of Environmental Health Sciences at the University of Massachusetts Amherst.

D. Alan Underhill, PhD
Associate Professor, Department of Oncology, University of Alberta

Dr. Alan Underhill is an Associate Professor in the Department of Oncology at the University of Alberta. He is the Director of the MD/PhD program in the Faculty of Medicine & Dentistry and President of the Canadian Society for Clinical Investigation (2015-2107). His research interests are in the area of genetic and epigenetic dysfunction in development and cancer. In particular, the Underhill lab studies protein and chromatin dynamics using a range of imaging platforms, as well as gene regulatory networks in melanocytes and melanoma using molecular and computational approaches.

CEEHRC Awardees

Tiago Medina, PhD
Postdoctoral Fellow, University Health Network

Alex Murison, PhD
Postdoctoral Fellow, University Health Network

Rapid Fire Talks

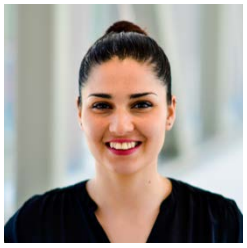
Alissa Cait
Graduate Student, Dept of Microbiology and Immunology
University of British Columbia

Sanaa Choufani, PhD
Research Associate
The Hospital for Sick Children

Elizabeth Chun
Graduate Student, Department of Medical Genetics
University of British Columbia

Shriya Deshmukh
Graduate Student, Division of Experimental Medicine
McGill University

Tony Hui
Graduate Student, Department of Microbiology and Immunology
University of British Columbia



Gurbet Karahan, PhD
Postdoctoral Fellow, Department of Human Genetics
McGill University

Ken Kron, PhD
Postdoctoral Fellow, Princess Margaret Cancer Centre
University Health Network

Romain Lambrot, PhD
Postdoctoral Fellow, Department of Animal Science
McGill University

Lauren Martin
Graduate Student, Department of Cellular and Physiological Sciences
University of British Columbia

Alain Pacis
Graduate Student, Department of Biochemistry
Université de Montréal

Julien Richard Albert
Graduate Student, Department of Medical Genetics
University of British Columbia

Kathryn Vaillancourt
Graduate Student, Department of Psychiatry
McGill University

Program Agenda

Sunday, November 26, 2017

1:00 pm - 4:00 pm	**Registration** *Foyer*

4:30 pm - 5:30 pm	**KEYNOTE PRESENTATION** *Emerald A/B* **Anne-Ferguson Smith (University of Cambridge)** *Variable silencing of the repeat genome - implications for non-genetic inheritance*

6:00 pm – 7:30 pm Canadian Science Publishing	**Cocktail Reception** *SLCC Squamish Lil'wat Cultural Centre, 4584 Blackcomb Way*

Monday, November 27, 2017

7:00 am - 8:20 am	**Breakfast** *Emerald A/B*

Session 1: High-Throughput Epigenome Mapping Chair: Martin Hirst *Emerald A/B*	
8:20 am - 8:30 am	**Open Meeting**
8:30 am - 9:00 am	Shyam Prabhakar (Genome Institute of Singapore) *Histone acetylome-wide association study of host response to tuberculosis infection*
9:00 am - 9:30 am	Jordana Bell (King's College London) *Epigenetics in twins*
9:30 am – 9:45 am	Maxwell Libbrecht (Simon Fraser University) *A unified encyclopedia of human functional DNA elements through fully automated annotation of 164 human cell types*
9:45 am - 10:00 am	Eric Campos (University of Toronto) *Histones and the Inheritance of Epigenetic Information*

10:00 am - 10:30 am	**Coffee Break** *Foyer*

Session 2: Mechanisms of Epigenomic Perturbation in Disease I Chair: Cheryl Arrowsmith *Emerald A/B*	
10:30 am - 11:00 am	Joe Costello (University of California San Francisco) *Evolution of IDH mutant brain tumors*
11:00 am - 11:30 am	Rosanna Weksberg (University of Toronto) *DNA methylation signatures for Mendelian disorders of the epigenetic machinery*
11:30 am – 12:00 pm	**RAPID FIRE TALKS** Alissa Cait (University of British Columbia) *An epigenetic link between the gut microbiome and immune responses in asthma* Sanaa Choufani (Hospital for Sick Children) *A Powerful Functional Tool for Assessment of Rare Germline Variants in the PRC2 Complex* Elizabeth Chun (University of British Columbia) *Extra-cranial rhabdoid tumours exhibit molecular similarities to the MYC-subgroup of AT/RTs* Shriya Deshmukh (McGill University) *Characterizing the Epigenetic Effects of the Histone H3.3 G34W Mutation in Giant Cell Tumors of Bone* Ken Kron (Princess Margaret Cancer Centre) *Noncoding somatic mutations modulate master transcription factor binding and cis-regulatory element activity in prostate cancer* Lauren Martin (University of British Columbia) *Generation and characterization of a novel mouse model of synovial sarcoma*

12:00 pm - 1:30 pm illumina	**Lunch Workshop** Steve Bruinsma, Product Development Scientist, Illumina *An EPIC tale: Targeted sequencing for DNA methylation* *Emerald A/B*

Data Visualization Workshop Chair: Steve Jones *Emerald A/B*	
2:00 pm - 3:30 pm	Martin Krzywinski (BC Cancer - Genome Sciences Centre) *Sense and Sensibility - Visual Design Principles for Scientific Data*

3:30 pm - 4:00 pm	**Coffee Break** *Foyer*

Session 3: Cellular Signaling Through the Histone Code I Chair: Guillaume Bourque *Emerald A/B*	
4:00 pm - 4:30 pm	Tatiana Kutateladze (University of Colorado) *Molecular mechanisms of epigenetic regulation*
4:30 pm - 5:00 pm	Mathieu Lupien (University of Toronto) *Metabolic adaptation as a driver of epigenetic reprogramming in cancer*
5:00 pm - 5:30 pm	**RAPID FIRE TALKS** Tony Hui (University of British Columbia) *Derivation and functional annotation of hematopoietic subpopulations from single-cell DNA methylation data* Gurbet Karahan (McGill University) *Interactions of paternal factors and the use of assisted reproductive technologies: effects on offspring and epigenetic outcomes* Romain Lambrot (McGill University) *Determining the role of histone methylation in human sperm* Alain Pacis (Université de Montréal) *Epigenetic regulation of innate immune responses* Julien Richard Albert (University of British Columbia) *De novo DNA methylation of paternal loci following fertilization - insights from a novel allele-specific pipeline for NGS datasets* Kathryn Vaillancourt (McGill University) *Methylation of the tyrosine hydroxylase gene (TH) in the striatum is associated with cocaine dependence in humans*

6:00 pm - 7:30 pm	**Dinner** *Emerald A/B*

illumina	**Poster Session 1** *Emerald C*
8:00 pm - 10:00 pm	See Poster Assignments (page 18)

Tuesday, November 28, 2017

7:00 am - 8:30 am	**Breakfast** *Emerald A/B*

Session 4: Regulation of the Epigenome During Development Chair: Nathalie Bérubé *Emerald A/B*	
8:30 am - 9:00 am	Wendy Robinson (University of British Columbia) *What can DNA methylation tell us about placental and fetal health?*

9:00 am - 9:30 am	Jacquetta Trasler (McGill University) *Similar impact of folic acid supplements on the sperm DNA methylome in mice and men*
9:30 am - 9:45 am	Richard Pilsner (UMass Amherst) *Identification of sperm DNA methylation loci associated with chronological age*
9:45 am - 10:00 am	Serge McGraw (Université de Montréal) *Early Embryonic Alcohol Exposure Leads to Permanent DNA Methylation Alterations in Mouse Brain and Placenta*

10:00 am - 10:30 am	**Coffee Break** *Foyer*

Session 5: Mechanisms of Epigenomic Perturbation in Disease II Chair: James Davie *Emerald A/B*	
10:30 am - 11:00 am	Jacek Majewski (McGill University) *Patterns of Chromatin Deregulation in Epigenetically Driven Cancers*
11:00 am - 11:30 am	Marco Gallo (University of Calgary) *Molecular links between self-renewal and chromatin dynamics in brain tumors*
11:30 am - 11:45 am	Jennifer Mitchell (University of Toronto) *Dissecting the function of enhancers at the nucleotide level with CRISPR/Cas9 to understand the mechanisms of disease*
11:45 am - 12:00 pm	Jean-Francois Couture (University of Ottawa) *Fine-tuning the implementation of H3K4 methylation by the DPY-30/Ash2L complex*

12:00 pm – 1:30 pm NEW ENGLAND BioLabs Ltd.	**Lunch Workshop** Feng Chan, Product Development Scientist, Cell Signaling Technologies *Analysis of Epigenetic Marks and Mechanisms: Your Guide to a Successful ChIP Assay* *Emerald A/B*

FREE TIME

3:30 pm - 5:30 pm	**Coffee Break** *Foyer*

NEW ENGLAND BioLabs Ltd.	**Poster Session 2** *Emerald C*
3:30 pm – 5:30 pm	See Poster Assignments (page 18)

Session 6: Cellular Signaling Through the Histone Code II Chair: F. Jeffrey Dilworth *Emerald A/B*	
5:30 pm - 6:00 pm	Benjamin Garcia (University of Pennsylvania) *Quantitative Proteomics for Understanding Modified Proteins and Proteomes*
6:00 pm - 6:30 pm	Anne-Claude Gingras (University of Toronto) *Bromodomain interactome profiling reveals complex modes of recruitment to BET proteins*
6:30 pm - 6:45 pm	Alan Underhill (University of Alberta) *Redefining the pericentromere through analyses of chromatin and protein dynamics*
6:45 pm - 7:00 pm	Michael Hendzel (University of Alberta) *Colour TEM reveals RNA to be an integral component of interphase chromatin*

7:30 pm - 9:30 pm	**Dinner** *The Brewhouse, 4355 Blackcomb Way*

Wednesday, November 29, 2017

7:00 am - 8:30 am	**Breakfast** *Emerald A/B*

Session 7: CEEHRC Awardees AGM Chair: Martin Hirst *Emerald A/B*	
8:30 am - 9:00 am	Presentation/updates from Mapping Centres Martin Hirst and Guillaume Bourque
9:00 am - 9:30 am	Tiago Medina (University of Toronto) *Microbial imbalance is associated with a distinct pro-inflammatory epigenomic and transcriptomic profile of intestinal CD4 T cells from Inflammatory Bowel Disease Patients*
9:30 am - 10:00 am	Alex Murison (University of Toronto) *Chromatin rearrangements observed over the earliest stages of hematopoiesis are co-opted to drive leukemogenesis in NPM1c Acute Myeloid Leukemia*
10:00 am – 10:15am	**Close Meeting**

10:30 am	**Bus departures**

Poster Assignments

Poster Session 1 Monday, November 27 8:00 – 10:00 pm		Poster Session 2 Tuesday, November 28 3:30 – 5:30 pm	
Number	**Name**	**Number**	**Name**
1	Karen Aitken	2	Bradley Balaton
3	Virginie Bertrand-Lehouillier	4	Steve Bilodeau
5	Darren Brenner	6	Julie Brind'Amour
7	David Bujold	8	Darci Butcher
9	Alissa Cait**	12	Eric Chater-Diehl
11	Donovan Chan	14	Haifen Chen
13	Carol Chen	15	Lu Chen
16	Sanaa Choufani**	18	Mackenzie Coatham
17	Elizabeth Chun**	22	Udit Dalwadi
20	Elodie Da Costa	24	Nicolas De Jay
21	Sarah Dada	25	Daniel Desaulniers
23	James Davie	28	Rachel Edgar
26	Shriya Deshmukh**	30	Erlinda Fernandez Diaz
27	Charles Dupras	32	William Gibson
29	Aida Eslami	34	Prerna Grover
31	France Gagnon	36	Munerah Hamed
33	David Gosselin	38	Alireza Heravi-Moussavi
35	Amanda Ha	40	Michael Hoffman
39	Geoff Hicks	42	Huayun Hou
41	Nadine Hosny El Said	44	Tony Hui**
43	Haihong Hu	46	Michael Johnston
45	Rashedul Islam	47	Gurbet Karahan**
48	Ken Kron**	49	David Labbé
52	Mackenzie Lawrence	50	Romain Lambrot**
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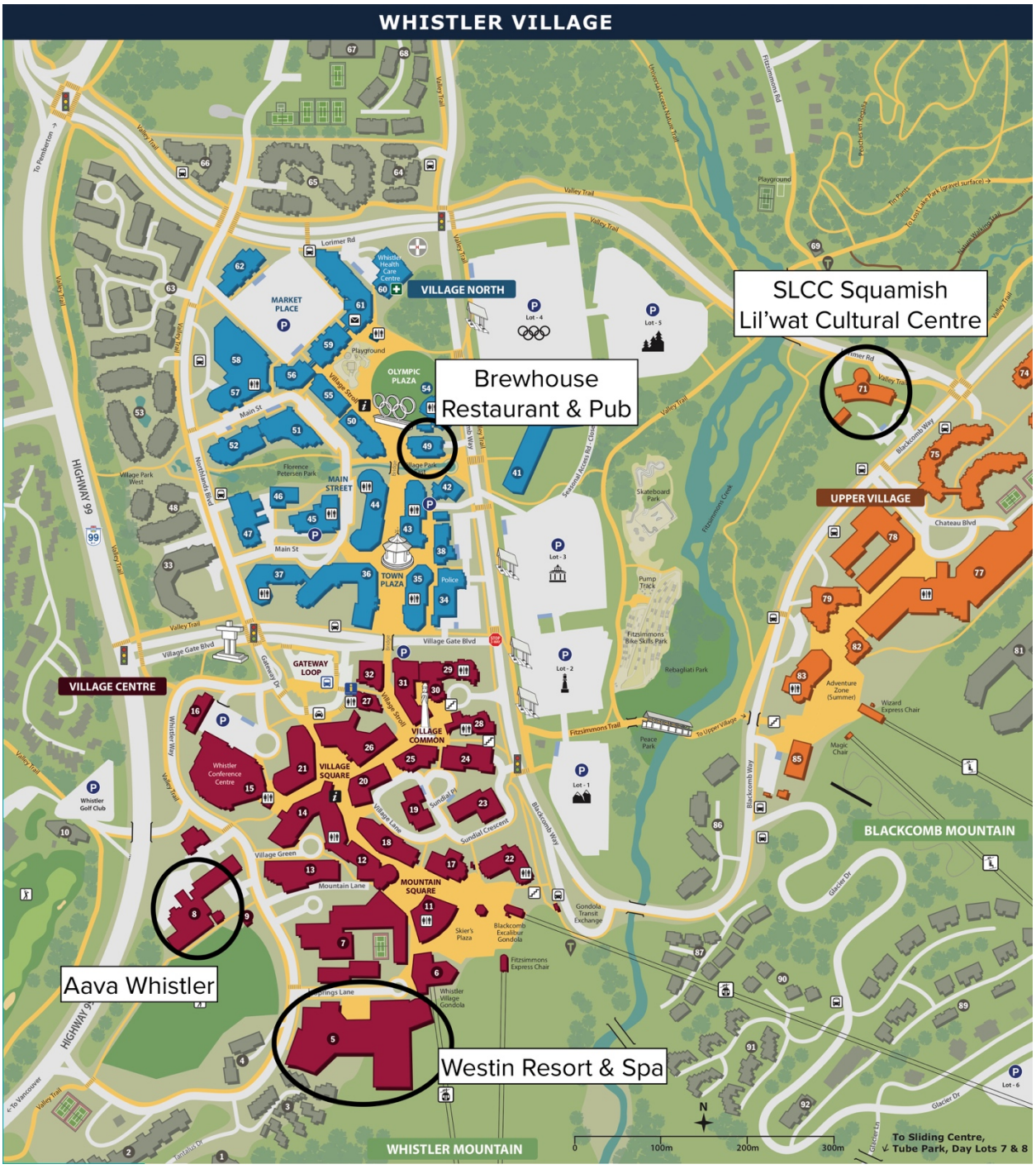

Abstracts
Selected Speakers

[60] A unified encyclopedia of human functional DNA elements through fully automated annotation of 164 human cell types

Maxwell W Libbrecht, Oscar Rodriguez, Zhiping Weng, Michael Hoffman, Jeffrey A Bilmes, William Stafford Noble

School of Computing Science at Simon Fraser University, British Columbia, Canada

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Unsupervised genome annotation methods such as Segway enable understanding of chromatin activity. These algorithms take as input a collection of genomics data sets and simultaneously partition the genome and label each segment with chromatin state label such that positions with the same label have similar patterns of activity.

We present chromatin state annotations of 164 human cell types using 1,615 genomics data sets. To produce these annotations, we developed a fully-automated annotation strategy in which we train separate unsupervised annotation models on each cell type and use a machine learning classifier to automate the state interpretation step. These annotations can be used to easily and intuitively look up the chromatin state of any given genomic position in any human tissue that has been profiled.

Using these annotations, we developed a measure of the functional importance of each genomic position called the "functionality score", which allows us to aggregate information across cell types into a multi-cell type view. This score provides a measure of importance directly attributable to a specific activity in a specific set of cell types. In contrast to evolutionary conservation, this measure is not biased to detect only elements shared with related species. Using the functionality score, we combined all our annotations into a single cell type-agnostic encyclopedia that catalogs all human functional regulatory elements, enabling easy and intuitive interpretation of the effect of genome variants on phenotype, such as in disease-associated, evolutionary conserved or positively selected loci.

These resources, including cell type-specific annotations, enyclopedia and a visualization server, are available online at http://noble.gs.washington.edu/proj/encyclopedia/ and on the UCSC genome browser.

[10] Histones and the Inheritance of Epigenetic Information

Eric I. Campos

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Chromatin is organized in nucleosomal particles, where DNA is wrapped around a histone octamer. The nucleosomal histone octamer is composed of a central (H3-H4)2 tetramer flanked by two H2A-H2B histone dimers. Histone proteins are subject to numerous posttranslational modifications, some of which are inherited throughout DNA replication. For these reasons histones are largely believed to propagate epigenetic information.

The replication machinery must be able to unwind and replicate DNA as histones dissociate and re-associate at replication forks. Despite a good understanding of how newly synthesized histones are deposited onto newly replicated DNA, we have yet to fully understand how existing nucleosomal histones segregate as they encounter replication forks. This is important given that existing 'parental' histones are believed to transmit epigenetic information each time a cell divides.

To gain a better understanding of how histones H3-H4 are chaperoned during DNA replication, we characterized protein-protein interactions that occur with histones, as well as with components of the replication machinery. Emphasis is here placed on the CDC45/MCM2-7/GINS (CMG) DNA helicase.

[78] Identification of sperm DNA methylation loci associated with chronological age

Haotian Wu[1], Alexander Suvorov[1], Cynthia Sites[2], Rahil Tayyab[2], J. Richard Pilsner[1]

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[2]Department of Obstetrics and Gynecology, Baystate Medical Center

Background. Paternal age has been associated with offspring disease susceptibility such as increased risk of schizophrenia, autism and some cancers. However, an understanding of the influence of age on sperm DNA methylation patterns is under investigated.

Objective. To identify differentially methylated sites and/or regions correlated with chronological age.

Methods. A total of 48 male participants were recruited from the Baystate Medical Center's IVF clinic from 2014 to 2015 as part of the Sperm Environmental Epigenetics and Development Study (SEEDS). Sperm DNA methylation was assessed with the HumanMethylation 450K array and relevant demographic and lifestyle information were collected via self-reported questionnaire. The raw 450K data was corrected by background fluorescence, probe type bias, and batch effects. The resulting data was analyzed via two methods. First, we used AClust algorithm to identify co-regulated regions, which were then modeled with age using linear generalized estimating equation models. Second, we used CpGassoc to model the association of age with all available CpG sites. All p-values were corrected for false-discovery rate via the Benjamini-Hochberg method. As a sub-analysis, we constructed models to predict age using CpG sites. We first selected CpGs that were statistically associated with age and ranked them by percent variance explained. We then used forward-selection to select a combination of 20 CpG sites that best predict age in our study.

Results. Of the 48 SEEDS males, 21% were < 30 years, 52% were 30-40 years and 27% were >40 years of age. DNA methylation of 1,170 CpG sites and 416 clusters were associated with age. In our prediction model, we identified 20 CpG sites that were able to explain nearly all of the variance in age (R^2 = 0.996). Gene ontology analyses of the genes associated with the 1,170 CpG sites revealed enrichment in glycoprotein, disulfide bond, cell membrane and topological domains (extracellular/intracellular).

Conclusions. We identified as few as 20 CpG sites that almost perfectly predicted chronological age in men seeking fertility treatment. Future studies will seek to validate these findings in additional IVF samples and from the general population as well as to determine the potential downstream effects of aging on biological pathways. We will also explore if methylation of these sites differ between fertile and infertile men.

[69] Early Embryonic Alcohol Exposure Leads to Permanent DNA Methylation Alterations in Mouse Brain and Placenta

Lisa-Marie Legault[1,2], Mélanie Breton-Larrivée[1], Virginie Bertrand-Lehouillier[1,2], Maxime Caron[1], Daniel Sinnett[1,2], Serge McGraw[1,2]

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Prenatal alcohol exposure is known to alter epigenetic profiles in cells during brain development and be part of the molecular basis underpinning Fetal Alcohol Spectrum Disorders (FASD) etiology. However, the consequences of a prenatal alcohol exposure during very early embryonic life on the future epigenetic landscape and developmental program regulating *cell* fate decisions (embryonic and extraembryonic) remain unknown. Our data indicate that prenatal alcohol exposure of early embryos increases rate of gross non-lethal abnormalities in embryos and placentas at mid-gestation. Further investigation using next-generation sequencing (RRBS: Reduced Representation Bisulfite Sequencing) revealed that alcohol exposure of preimplantation embryos, during the epigenetic reprograming wave, leads to permanent alterations in the future epigenetic program of brain and placenta cells. Surprisingly, the exposure leads to long-lasting sex-specific differences in DNA methylation alterations between male and female samples. Our results also suggest that abnormal epigenetic profiles observed in the placenta could potentially be use as a non-invasive predictive test offered to mothers that inadvertently subjected their early embryos to acute levels of alcohol and believed that their newborn child is at risk for FASD. Our research will make a significant step toward deciphering the molecular basis underlying early embryonic onset of FASD by determining how alterations in the embryo's epigenetic program ultimately leads to aberrant regulation of gene expression and impaired brain development.

[71] Dissecting the function of enhancers at the nucleotide level with CRISPR/Cas9 to understand the mechanisms of disease.

Virlana M Shchuka, Gurdeep Singh, Sakthi D Moorthy and Jennifer A Mitchell

Department of Cell and Systems Biology, University of Toronto, Toronto, ON, M5S 3G5, Canada

Genome-wide association studies (GWAS) have revealed that close to 80% of single nucleotide polymorphisms (SNPs) associated with disease are found within the non-coding regions of the genome that display chromatin modifications consistent with enhancer (ENH) activity. Often multiple enhancers are associated with the regulation of a single gene, with these enhancers clustered in the linear genome into what have been recently termed super-enhancers. Using CRISPR/Cas9 genome engineering we investigated the function of several gene regulatory regions in embryonic stem cells by deleting individual enhancers or entire enhancer clusters. This approach showed that individual enhancers within an enhancer cluster are completely or partially redundant, with the deletion of a single enhancer affecting transcriptional output by less than 50%. At the same time, we identified gene clusters regulated by single isolated enhancers such that deletion of one enhancer disrupted >80% of the transcriptional output of multiple genes. At the nucleotide level, individual enhancers contain binding motifs conserved through evolution for on average seven different transcription factors. These transcription factor binding motifs function in a synergistic manner as the loss of any single motif greatly affects enhancer activity. Clustered enhancers, however, buffer the effect of disrupting one transcription factor binding motif as individual enhancers within the cluster are partially or completely redundant. By contrast, transcription of genes controlled by single enhancers is more susceptible to single nucleotide changes that disrupt transcription factor binding and enhancer function. Together these data revealed that super-enhancers do not regulate higher transcriptional output of their target genes but instead provide robustness in regulatory control that can buffer the effect of single mutations which might otherwise greatly disrupt target gene transcription. The subtle changes in gene transcription that we observed after disrupting one enhancer within a clustered enhancer could explain the relatively subtle effect most SNPs have on disease risk.

[19] Fine-tuning the implementation of H3K4 methylation by the DPY-30/Ash2L complex

Jean-Francois Couture

Department of Biochemistry, Microbiology and Immunology, University of Ottawa

Mixed Lineage Leukemia (MLL) proteins are part of a family of enzymes that play a critical role during embryonic development and are now known to be heavily mutated in various types of cancers. These enzymes are systematically found associated with various proteins and when assembled in multi-subunit complexes, they are referred to as COMPASS-like complexes. Among these regulatory proteins, a four-subunit complex composed of WDR5, RbBP5, Ash2L and DPY30 (also referred to as WRAD) allosterically regulates the methyltransferase activity of MLL proteins. Collectively, these subunits form a platform that stimulates the methyltransferase activity of MLL1 as well as integrates biological cues to modulate the functions of this TrxG protein. Detailed genome-wide, molecular and structural studies reveal that an extensive network of interactions takes place at the interface between Dpy-30 and Ash2L. These interactions are critical for the correct positioning of the yeast MLL-like complex at transcriptional start sites and the genome wide localization of H3K4me2 and H3K4me3. Moreover, these interactions are essential for the implementation of H3K4 methylation in leukemic cells. Finally, we show that a novel inhibitor targeting the formation of the DPY-30/Ash2L sub-complex efficiently block H3K4 methylation as well as gene expression in cells and displays pharmacological activities in blocking the progression of breast tumors in mice. Overall, our findings provide structural insights into the assembly of the WRAD complex and point to novel regulatory paradigms controlling the activity of the KMT2/COMPASS family of lysine methyltransferases.

[103] Redefining the pericentromere through analyses of chromatin and protein dynamics

Hilmar Strickfaden, Kristal Missiaen, Michael J. Hendzel and D. Alan Underhill

Department of Oncology/Division of Experimental Oncology, Cross Cancer Institute, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada

The centromere of eukaryotic chromosomes is essential to safeguarding genome content during cell division and its dysfunction leads to pathogenic conditions, including birth defects and cancer. It is observable as the constriction between sister chromatids during mitosis and comprises 2 subdomains, the inner centromere and pericentromere, which are critical for kinetochore assembly and chromatid cohesion. Despite their contiguous chromosomal location, these subdomains form discrete compartments throughout the cell cycle, which are defined epigenetically by distinct chromatin features and associate proteins. For the pericentromere, this reflects trimethylation of histone H3-lysine 9 (H3K9me3) and histone H4-lysine 20 (H4K20me3) via the action of the CBX5 chromobox protein and the SUV39H1/2 and SUV420H1/2 lysine methyltransferases (KMTs). In this context, CBX5 is reported to drive phase separation to partition these pericentromeric components in a manner similar to how oil forms droplets in water. Although this creates a barrier to protein entry and exit, it is dynamic and the entire CBX5 population within the pericentromere turns over in seconds by exchange with the surrounding nucleoplasm. Here, we define a novel biological state for SUV420H2 where it is mobile within the pericentromere but undergoes very limited nucleoplasmic exchange. This reflects a 20-fold difference in partition coefficients between SUV420H2 and CBX5, as well as reduced diffusion when assessed by fluorescence-correlation spectroscopy, establishing the former as a more stable component of the pericentromere. Interestingly, this behavior is conferred by a discrete region in SUV420H2 outside of the catalytic domain and is highly dependent on the underlying chromatin state, suggesting it may function as a novel chromatin recognition module. Intriguingly, it can also induce ectopic DAPI-foci in human cells and therefore appears to have an inherent ability to promote pericentromere and/or heterochromatin clustering, which itself may reflect phase separation. Finally, although SUV420H2 is thought to be recruited to the pericentromere by CBX5, mutational analyses suggest a more complex mechanism that supports the presence of additional localization determinants. Nevertheless, co-expression of SUV420H2 and CBX5 attenuates the mobility of both proteins, suggesting they may undergo further phase transitions to gel or solid-like states. Overall, these findings represent a fundamental change to models of pericentromere organization and create a framework for understanding its normal and aberrant function.

Funding for this project: Canadian Breast Cancer Foundation-Prairies/NWT, Women & Children's Hospital Research Institute (University of Alberta), and Alberta Cancer Foundation.

[37] Colour TEM reveals RNA to be an integral component of interphase chromatin

Hilmar Strickfaden, Darin McDonald, and Michael J Hendzel

Departments of Oncology and Cell Biology, Faculty of Medicine and Dentistry, University of Alberta

We have previously used electron spectroscopic imaging (ESI) to visualize and quantify the composition of nucleoprotein and protein structures of the interphase nucleus. This two colour technique has proven very useful for studying changes in chromatin organization at the nanoscale in situ. We have been expanding on this technology with the objective of using ESI to develop a multichannel colour transmission electron microscopy (cTEM) technology with capabilities similar to fluorescence microscopy. We will present progress towards that goal with an emphasis on our results developing an approach to study the relationship between chromatin and RNA. Conventional TEM approaches have been able to selectively stain RNA or DNA but it has not been possible to visualize both while distinguishing the two with certainty. RNA was reported to be found in three nucleoplasmic structures: perichromatin fibrils, perichromatin granules, and interchromatin granules. ESI revealed only perichromatin granules and interchromatin granules. Chromatin was defined as any fibrillar structure in the 5-25 nm diameter range found in the nucleoplasm and highly enriched in phosphorus atoms. There was no apparent equivalent to what had been described as perichromatin fibrils. Rather, our interpretation was that the previous techniques misidentified fibrillar protein structures as RNA. Our interpretation was consistent with the rapid RNA folding into granules previously reported for the only gene to be successfully visualized in the process of transcription—a Balbiani ring gene of Chironomus tentans.

Nonetheless, a major limitation of our previous approach is that we could not definitively distinguish between RNA-based fibers and DNA-based fibers because both are phosphorus rich. We have now developed an approach that has allowed us to generate colour TEM data that enables the simultaneous visualization and discrimination of RNA from DNA. Using this approach, we have validated the presence of what were previously described as perichromatin fibrils. Surprisingly, our results reveal that our previous ESI work misidentified perichromatin fibrils as 10 nm chromatin. We also find that the “perichromatin” assignment reflected the fact that chromatin could only be identified by an absence of stain in the conventional TEM approach for visualizing RNA. Visualizing the DNA as a separate colour enabled us to directly assess the relationship with chromatin. Instead of being exclusively perichromatin, we find that RNA is integral to both heterochromatin and euchromatin higher-order structures. Our results suggest that RNA is far more important to the organization of interphase chromatin than currently appreciated.

Abstracts
Rapid Fire Talks

[9] An epigenetic link between the gut microbiome and immune responses in asthma

Alissa Cait, Michael R. Hughes, Misha Bilenky, Jessie Cait, Kelly M. McNagny, Martin Hirst, William W. Mohn

The Biomedical Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

Asthma has become the most common childhood disease in developed countries, with major social and economic consequences. The causes of asthma are complex and poorly understood, but there is a growing body of evidence that implicates dysbiosis of the gut microflora as a driving force behind the development and severity of asthma and allergy. In infants, we previously found an association between the gut microbiome and asthma. We have now found that vancomycin treatment of mice causes gut dysbiosis marked by a selective loss of fibre-fermenting taxa leading to an altered metabolome with reduced production of the short chain fatty acid (SCFA) metabolite butyrate. We found that mice on vancomycin, thus lacking exposure to butyrate, have a pro-inflammatory immune phenotype with heightened TH2 response in a model of asthma. We subsequently found that exogenous supplementation of SCFA to vancomycin-treated mice did not significantly alter the gut dysbiosis but was sufficient to ameliorate allergic severity due to vancomycin. SCFA are known to exert their effects on target cells predominantly through two mechanisms: G-protein coupled receptors and inhibition of histone deacetylases (HDAC). Butyrate is the most potent SCFA-based inhibitor of HDACs. Moreover, because HDAC activity modifies the epigenetic state of the genome, the effects of a butyrate-HDAC axis are likely to promote long term changes in gene expression and cell fate. To investigate the long-term effects of butyrate exposure on the hematopoietic compartment, we successfully transferred the phenotypes of both heightened and dampened TH2 skewing via bone marrow (BM) transplants to irradiated recipient mice. Consistent with the hypothesis that the transferred phenotype is encoded within the epigenome, we found unique regulatory states, as defined by H3K27ac, within the genomes of purified hematopoietic stem and progenitor cells of mice that received BM transplants from dysbiotic mice. Differentially expressed genes and pathways were dominated by immune functions up-regulated in dysbiotic mice. This work provides understanding of a novel epigenetic process linking the microbiome and the immune system and has potential practical applications in the prevention and treatment of asthma.

[16] A Powerful Functional Tool for Assessment of Rare Germline Variants in the PRC2 Complex

Sanaa Choufani[1], Ana Cohen[2], Cheryl Cytrynbaum[3], Tianren Wang[1], Lucie Dupuis[3], Roberto Mendoza-Londono[1,3], Brian HY Chung[4], David Chitayat[3,5], Jerry Machado[6], Kate Tatton-Brown[7], William T. Gibson[2], Rosanna Weksberg[1,3,8].

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Polycomb Repressive Complex 2 (PRC2) plays an essential role in chromatin modification by methylating Lys 27 of Histone H3 (H3K27me3). The PRC2 core is composed of four core subunits, EZH2/1, SUZ12, EED, and RBAP46/8. The catalytic subunit is the SET domain-containing protein EZH2. Several of the SET proteins have been identified as the cause of genetic disorders such as Weaver syndrome (WS) and other related overgrowth syndromes. We and others have shown that germline mutations in chromatin-modifying enzymes (epigenes) known to be associated with serious developmental problems display a specific DNA methylation (DNAm) signature that overlaps pathways functionally important for the maintenance and regulation of specific transcriptional states throughout development. WS characterized by overgrowth and intellectual disability, is caused by mutations in *EZH2*, an epigene encoding a histone methyltransferase. Recently WS-like features were also found to be caused by mutations in *EED* and *SUZ12* suggesting that mutations in any of the 3 genes forming the PRC2 complex can cause WS. We profiled DNAm from lymphocytes of 40 individuals with mutations in the PRC2 complex from clinicians worldwide and over 200 controls using the Illumina methylationEPIC arrays. Using Statistical tools available in R and the epigenetic analysis pipeline developed in the Weksberg lab, we were able to show that pathogenic mutations in *EZH2* are associated with DNAm changes supporting a role for EZH2 in controlling DNAm through direct physical contact with DNA methyltransferases. We found that differentially methylated CG sites overlap with regions of dense unmethylated DNA, CpG islands which are known Polycomb target sites. In addition, we showed that mutations in *SUZ12* and *EED* share the same DNAm targets as *EZH2* highlighting the fact that pathogenic variants in *EZH2*, *EED* and *SUZ12* generate a common DNAm signature likely reflecting their functional partnership in the PRC2 complex. Also, these findings support the concept that WS is a single disorder with locus heterogeneity. Finally, we were able to show that putative gain of function (GOF) mutation in *EZH2* with an opposite clinical presentation (undergrowth) generate a DNAm profiles opposite to the one with loss of function mutations (LOF) in the PRC2 complex demonstrating the powerful potential of DNAm signatures in the assessment of rare germline epigene mutations. *In silico* analysis revealed that the PRC2 signature is highly enriched in promoter regions of certain transcription factor genes involved in development as well as genomic regions that are associated with EZH2 binding in embryonic stem cells. Novel PRC2-specific DNAm signature will have the capacity to support Weaver syndrome diagnoses, stratify non-synonymous variants in the PRC2 complex genes as benign or pathogenic, LOF or GOF, and elucidate the molecular mechanisms underlying the pathophysiology of WS.

[17] Extra-cranial rhabdoid tumours exhibit molecular similarities to the MYC-subgroup of AT/RTs

Hye-Jung E Chun[1], Pascal D Johann[2], Mikhail Bilenky[1], Emilia Lim[1], Alireza Heravi-Moussavi[1], Dean Cheng[1], Young Cheng[1], Tina Wong[1], Eric Chuah[1], Nina Thiessen[1], Yussanne Ma[1], Daniela S Gerhard[3], Andrew J Mungall[1], Richard A Moore[1], Steven JM Jones[1,4,8], Elizabeth J Perlman[5]; Martin Hirst[1,6], Annue Huang[7], Marcel Kool[2], Marco A Marra[1,8]

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Rhabdoid tumours (RTs) are lethal pediatric cancers that account for nearly 10% of all cancer cases in infants. Currently, RTs are broadly classified based on tissue types of occurrence i.e. cranial Atypical Teratoid (AT) RTs and extra-cranial malignant RTs (ecRTs). Regardless of the site of occurrence, >95% of RTs harbour loss of SMARCB1, a subunit of the chromatin-remodeling SWI/SNF complex, and are reported to share similar histological characteristics. Molecularly, RTs are heterogeneous. Previous studies reported molecular subgroups within RTs of the same tissue type. However, the extent to which RT subgroups of different tissue types are related and molecular characteristics shared among them remains unknown. To address how RTs from different anatomic sites are similar, we compared DNA methylation and gene expression profiles of 140 ecRTs and 161 AT/RTs using WGBS or 450K/850K arrays and RNA-Seq. To assess molecular similarities, we performed unsupervised clustering using DNA methylation data and found that ecRTs clustered with the MYC-subgroup of AT/RTs while the SHH- and TYR-subgroups of AT/RTs clustered separately. Over-expression of *c-MYC* and the non-coding regulatory RNA gene *HOTAIR*, characteristic of MYC-AT/RTs, was also observed in ecRTs. ecRTs and MYC-AT/RTs further exhibited over-expression of genes enriched for development of mesenchymal cell types such as skeletal, vasculature, and muscle systems. In contrast, non-MYC-AT/RTs exhibited over-expression of genes enriched for neural development including axon guidance and neuron differentiation. Our results indicate molecular similarities between ecRT and MYC-ATRTs and distinct developmental processes associated with them compared to other AT/RT subgroups.

[26] Characterizing the Epigenetic Effects of the Histone H3.3 G34W Mutation in Giant Cell Tumors of Bone

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Introduction: Pediatric glioblastomas (pGBM) are malignant brain tumors associated with a dismal prognosis. A subset of pGBMs carry mutations of either the Lysine 27 or Glycine 34 (G34) amino acid residues of histone 3 variant genes. The same G34 residue is also mutated in 85-95% of giant cell tumors of bone (GCTs); albeit to Tryptophan (G34W) in GCTs rather than to Arginine or Valine (G34R/V) as in pGBMs. Mutations of the G34 residue are predicted to impede access of histone methyltransferases like SETD2 to the adjacent Lysine 36 residue, thereby altering the epigenome and transcriptome.

Methods: To elucidate the tumorigenic effect of G34 mutations, we used the gene-editing technology CRISPR/Cas9 to correct the G34W mutation to wildtype in two GCT cell lines. We then characterized CRISPR-edited cell lines using functional assays, proteomic, epigenomic and transcriptomic analyses.

Results: Correction of the G34W mutation to wildtype in GCTs results in reduced proliferation rate and colony formation. By mass spectrometry, G34W GCT cell lines display decreased levels of Lysine 36 trimethylation (H3K36me3) on the mutant G34-peptide, similar to pGBM cell lines carrying G34R/V mutations. However, unlike pGBMs, GCTs display increased levels of Lysine 36 dimethylation (H3K36me2) on the mutant G34-peptide. Unsupervised hierarchical clustering of RNA-Seq data reveals that the G34W parent GCT clusters independently from CRISPR-edited wildtype clones, indicating a distinct gene expression profile driven by the G34W mutation.

Ongoing experiments: We are currently comparing the level and distribution of H3K36me2 and H3K36me3 marks by performing chromatin immunoprecipitation followed by next-generation sequencing (ChIP-Seq) of CRISPR-edited wildtype clones relative to the parent G34W GCT cell line.

Conclusion: The G34W-mutation clearly has an impact on tumorigenic potential, as evidenced by *in vitro* functional assays. The G34W-mutant peptide of GCT cell lines features a distinct set of post-translational histone modifications compared to the G34-mutant peptide of pGBM cell lines. In addition, the G34W mutation drives a specific gene expression profile. Further investigation could elucidate the epigenetic mechanism(s) through which the G34W mutation confers its tumorigenic properties.

[48] Noncoding somatic mutations modulate master transcription factor binding and cis-regulatory element activity in prostate cancer

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Prostate cancer (PCa) is the most common male malignancy in the western world. Single nucleotide mutations affecting protein-coding genes, such as those found in *SPOP*, *FOXA1*, or *TP53*, are less common than recurrent structural rearrangements and copy number aberrations in PCa. The extent to which the non-coding, cis-regulatory landscape is affected by functional single nucleotide variants (SNVs), however, remains poorly characterized. To assess the role of SNVs within cis-regulatory elements, we integrated whole genome sequencing data from 200 PCa patients with H3K27ac ChIP-seq data also obtained from primary prostate tumours. We show that mutations enrich within the binding sites of transcription factors (TFs) that play key roles in PCa, including FOXA1, AR, and HOXB13. A number of these mutations that are either directly within a TF binding motif or flanking it are predicted to impact the affinity of these factors to DNA. CRISPR-Cas9 mediated functional validation reveals that mutations within TF binding sites at PCa risk associated loci favour the enhancement of cis-regulatory element activity and MYC oncogene expression. Furthermore, we identify >1000 significantly mutated cis-regulatory elements throughout the genome, and characterize the role of such mutations in reducing expression of the potent prostate tumour suppressor ZFHX3. Taken together, our work highlights the extent to which cis-regulatory elements are targets of mutational forces and delineates a hitherto underappreciated driving force in oncogenic gene expression changes in prostate cancer.

[67] Generation and characterization of a novel mouse model of synovial sarcoma

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Synovial sarcoma is an aggressive soft-tissue sarcoma that most frequently affects adolescents and young adults and can arise in virtually any area of the body. The biologic behaviour of synovial sarcomas can vary depending on a variety of factors including histologic subtype, tumour size and age at diagnosis, and the 10-year survival rate has been estimated at 50%. Synovial sarcoma is characterized by a t(X;18)(p11.2;q11.2) translocation which produces a fusion oncogene named SS18-SSX. The SS18-SSX fusion oncoprotein is believed to alter transcriptional regulation by associating with the mSWI/SNF chromatin remodeling complex and the Polycomb group of transcriptional repressors leading to aberrant gene expression. It has been previously shown that SS18-SSX disrupts the mSWI/SNF complex and causes ejection and subsequent degradation of another mSWI/SNF subunit, BAF47, loss of which is observed in other types of aggressive neoplasms including epithelioid sarcoma and malignant rhabdoid tumour. The cell of origin of synovial sarcoma is currently unknown, although recent research indicates that it may arise from a mesenchymal stem/progenitor cell (MSPC) population.

We have generated a unique mouse model of synovial sarcoma where the human SS18-SSX fusion oncogene is conditionally expressed in a population of MSPCs along with an EGFP marker. This model shows robust formation of synovial sarcomas with a latency period of 16 weeks. Histologic analysis of tumours reveals characteristic cellular morphology and immunofluorescent staining confirms expression of common markers of synovial sarcoma. Preliminary RNA-seq analyses show that gene expression in these tumours parallels transcript profiles observed in human synovial sarcoma samples. Importantly, MSPCs from control animals are being used as a normal comparator to enable identification of the epigenetic differences between normal and neoplastic cells.

This new mouse model effectively recapitulates synovial sarcoma tumours found in humans and suggests that this population of MSPCs is a likely candidate for the cell of origin of this neoplasm. The robustness and reproducibility of this model provides us with a unique opportunity to elucidate the epigenetic changes underlying synovial sarcomagenesis.

[44] Derivation and functional annotation of hematopoietic subpopulations from single-cell DNA methylation data

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Hematopoietic stem cells (HSC) are heterogeneous in their ability to durably reconstitute the blood system. Epigenomic surveys along the hematopoietic hierarchy and within HSC compartments have revealed distinct epigenomic states associated with functionally defined hematopoietic cell types. However, our understanding of the degree to which epigenetic heterogeneity is related to repopulation capacity of a cell type is still incomplete. To address this, we performed single-cell genome-wide DNA methylation profiling of 148 murine and 121 human primary hematopoietic stem cells purified from adult mouse bone marrow and human cord blood. For this we developed a protocol that had a higher library diversity than existing protocols which in turn allowed for more comprehensive single-cell coverage (~4.5 million CpGs per cell). We utilized a novel analytical strategy that considers the methylation differences at single CpG sites across single-cells. With deeper sequencing, we regenerated near complete *in silico* bulk profiles of single-cell subpopulations from just 6 single-cells. We identified epigenetically distinct subpopulations of cells present in murine and human HSCs roughly corresponding to their respective repopulation potentials. Within mouse samples, enrichment analysis of genes associated with this distinct epigenetic state revealed pathways consistent with hematopoietic stem cell function, and integration with single-cell RNA-seq data from murine HSCs defined 22 putative surface proteins that may mark this population including the previously identified hematopoietic marker CD82. In humans, we show that CpG methylation can accurately separate hematopoietic stem cells from more differentiated progenitors. Furthermore, gene enrichment association with leukemic pathways suggests that a subpopulation within human HSCs may have both high self-renewal and differentiation potential and thus may be the population that can durably repopulate a recipient mouse.

[47] Interactions of paternal factors and the use of assisted reproductive technologies: effects on offspring and epigenetic outcomes

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Proper DNA methylation of sperm is indispensable for fertility and healthy offspring. There is increasing evidence that the sperm epigenome is affected by many factors including diet, smoking and toxicants and that abnormal epigenetic patterns can be transmitted to offspring. Not only environmental factors but also genetic factors can influence the sperm epigenome. An epigenetic regulator, DNA methyltransferase-3 like (DNMT3L), indirectly participates in *de novo* methylation in developing germ cells and is essential for spermatogenesis. Over the last few decades, there has been an increase in the use of assisted reproductive technologies (ART). Although ART is generally accepted as safe, research indicates an increase in birth and imprinting defects with ART. Our aim was to understand how paternal factors (obesity and/or *Dnmt3L* haploinsufficiency), in combination with ART, adversely influence offspring development and DNA methylation patterning. Eight-week old *Dnmt3L*$^{+/-}$ and wildtype *Dnmt3L*$^{+/+}$ C57BL/6 male mice were fed either a matched control diet (10% kcal, Ctrl) or a high fat diet (60% kcal, HFD) for 12 weeks. At the end of treatment, sperm were used to generate embryos by *in vitro* fertilization (IVF) and DNA was extracted for methylation analysis using reduced representation bisulfite sequencing (RRBS). Following ART, embryos and placentas were collected at mid-gestation (E11.5) to assess embryonic development. Males in the HFD group gained ~25% more weight and showed higher rates of subfertility than those in the Ctrl group. Analysis of pregnancy outcomes post ART did not indicate a significant difference in pre-implantation loss and litter sizes between diets or genotypes. However, a significant increase was observed in post-implantation loss following IVF with sperm from HFD males. A wide range of embryonic abnormalities that have previously been found following mouse ART (craniofacial, laterality defect, neural tube etc.), was observed in all groups at similar levels. Furthermore, the percentage of delayed embryos was not significantly different between groups. An initial examination of sperm DNA methylation on a genome-wide level by RRBS indicated hypomethylation of sperm DNA in the *Dnmt3L* $^{+/-}$ males, an effect that was amplified in HFD mice. Together these results indicate that the effects of paternal *Dnmt3L* haploinsufficiency on sperm DNA methylation and offspring outcomes following ART are exacerbated by the fathers' preconception consumption of high fat diets, providing evidence of a gene-environment interaction. (Supported by CIHR).

[50] Determining the role of histone methylation in human sperm

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Effects of the paternal environment including stress, diet, and toxicants have been linked to negative outcomes for offspring including birth defects and increased risks for complex diseases. These paternal effects may occur via non-genetic inheritance, through epigenetic mechanisms including DNA methylation, post-translational modifications of histones and noncoding RNAs. In mice, we recently showed that disruption of histone methylation in developing sperm has transgenerational consequences for the health and development of offspring (Siklenka et al, Science, 2015). Whether there are similar responses in men is unknown. Therefore, there is an immediate need to investigate the role of variation in sperm histone methylation in infertility and the reproductive outcomes of men. Our objective was to assess the differences in tri-methylation of the histone H3 on lysine 4 (H3K4me3) between fertile and infertile men. Methods: Sperm was collected from study participants at CReATe fertility centre in Toronto. ChIP-seq targeting H3K4me3 was performed on individual patients with normal sperm parameters that had produced offspring successfully (fertile, n=18) or not (infertile, n=18). Results: Our bioinformatic analysis revealed a great similarity of the H3K4me3 profiles between the fertile men, indicating the reproducibility of our ChIP-seq experiments. Remarkably, using the Bioconductor *csaw*, we observed differences in the H3K4me3 enrichment at 3600 regions between the fertile and infertile samples. These regions were located in genes implicated in development, cell cycle regulation, differentiation, and spermatogenesis. In our ongoing study, we are trying to further highlight the changes induced by the paternal environment (e.g. BMI, folate status...) on the sperm H3K4me3 pattern. These findings suggest that the sperm epigenome may play a crucial role in male fertility and influence the health and development of offspring.

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[75] Epigenetic regulation of innate immune responses

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Innate immune cells, such as dendritic cells (DCs) and macrophages, are the first actors recruited to respond to an invading pathogen. Upon stimulation, these cells undergo pervasive changes in their transcriptional program, mobilizing hundreds of genes involved in immune-related processes in a rapid and highly choreographed fashion. This is achieved with the help of signal-dependent transcription factors (TFs), including NF-κB/Rel, AP-1, and interferon regulatory factors (IRFs), which bind to gene regulatory regions of the genome where they initiate recruitment of various co-activators. Epigenetic processes, such as DNA methylation, are recognized as important permissive or suppressive factors playing an important role in modulating the access of TFs to cis-acting enhancer elements by regulating chromatin dynamics, therefore having a significant impact in gene expression. Indeed, previous work has shown that the infection of post-mitotic DCs with Mycobacterium tuberculosis (MTB), is associated with loss of methylation at enhancers and that such changes are strongly predictive of changes in the expression levels of nearby genes. To date, many other studies correlate these two processes, but it remains unclear whether altered methylation patterns themselves invoke transcriptional modulation or are instead downstream consequences of regulatory factors. Thus, the causal relationship between changes in DNA methylation and gene expression during infection remains to be elucidated.

Here we characterized genome-wide patterns DNA methylation, gene expression and chromatin accessibility in non-infected and MTB-infected DCs at multiple time points (2, 18, 48 and, 72 hours). We found that immune responses to infection are accompanied by stable loss of methylation at thousands of CpG sites overlapping distal enhancer elements. Interestingly, differentially methylated sites were highly enriched for 5-hydroxymethylcytosine (5hmC) in non-infected DCs. However, differential methylation at these loci was undetectable at 2 hours post-infection, even though a significant proportion of their target genes were already differentially expressed. Indeed, footprinting analysis revealed that NF-κB/Rel transcription factors were recruited to these regulatory regions, which led to chromatin decondensation and subsequent gain of H3K27ac activating mark in these regions, prior to loss of methylation. Although NF-κB/Rel occupancy sites were unaffected by DNA methylation, we observed specific cases of transcription factors that only bind after loss of methylation (at 18 hours or later).

These results suggest that although changes in methylation are downstream consequences of responses to infection, it is possible that these changes may be important for the binding of methylation-sensitive TFs. Furthermore, the enrichment of 5hmC as stable epigenetic modification at differentially methylated sites prior to infection suggests its importance in priming enhancers prior to activation. Our observations yield unprecedented insights on the hierarchy of DNA methylation in the control of transcriptional responses to infection.

[84] *De novo* DNA methylation of paternal loci following fertilization - insights from a novel allele-specific pipeline for NGS datasets

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Spermatozoa and oocytes have distinct chromatin states including unique DNA methylation (DNAme), histone post-translational modification (PTM) and transcription profiles, which are reprogrammed immediately following fertilization. Indeed, the hypermethylated sperm genome undergoes global demethylation in the one cell zygote, while the moderate methylation levels in the oocyte are maintained on the maternal genome at this stage. While the inheritance of paternal epigenomic marks during early zygotic development has been reported using microscopy-based immunofluorescence (IF) approaches, these are inherently of low resolution and therefore uninformative with respect to specific genomic loci. Further, while such studies have revealed large-scale epigenomic dynamics, dissecting the interplay between local DNAme, histone PTMs and transcription is not possible using IF.

To measure epigenome remodeling in the developing zygote at high resolution, we developed ALEA2, a computational pipeline for integrated allele-specific profiling of NGS-based DNAme, histone PTMs and transcription datasets. Utilizing publicly available whole genome bisulphite sequencing in gametes and F1 hybrid 2-cell mouse zygotes to track DNAme dynamics with single nucleotide resolution, we observe a genome-wide decrease of 43% and 7% DNAme on the paternal and maternal genomes, respectively, as expected. Interestingly, 17% of the paternal genome displays high DNAme at the 2C stage, and 77% of these regions retain DNAme until the blastocyst stage, suggesting a mechanism for DNAme protection similar to the one described for the maternal genome may be acting on a subset of the paternal genome. Furthermore, in spite of the wave of global paternal genome demethylation, we uncover robust DNAme gain at 3% of all hypomethylated spermatozoa loci, including at transcription start sites (TSSs). Half of these antithetical loci maintain such DNAme at the blastocyst stage, suggesting they may be involved in coordinating proper preimplantation development. Indeed, compared to TSSs that do not gain paternal DNAme, TSSs that gain paternal DNAme immediately following fertilization display reduced gene expression in preimplantation zygotes. Further analyses of allele-specific histone PTMs, chromatin-chromatin interactions and transcription at these paradoxical *de novo* methylated loci will be presented.

These results demonstrate the value of enhancing the level of resolution in NGS dataset analyses and reflect the potential for future discoveries using ALEA2.

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[104] Methylation of the tyrosine hydroxylase gene (*TH)* in the striatum is associated with cocaine dependence in humans.

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Background: Multiple, interaction biological mechanisms are likely to contribute to the development and maintenance of cocaine use disorders. Of particular interest is DNA methylation as it may mediate the long-term effects of chronic cocaine abuse on brain cell functioning. While research has identified numerous sites of hyper- and hypo-methylation in rodents, little is known about the relationship between cocaine dependence and DNA methylation in the context of the human disorder.

Methods: We used Reduced Representation Bisulfite Sequencing (RRBS) to identify differentially methylated region (DMRs), in striatal brain tissue from 25 individuals with cocaine dependence and 25 drug-naïve controls. We focused on two addiction-relevant striatal sub-regions, the nucleus accumbens (NAc) and caudate nucleus (CD), and validated a DMR within the tyrosine hydroxylase (*TH*) gene using targeted amplicon bisulfite sequencing. We used this method on an independent cohort of human samples (n=18 per group) and on neuronal and non-neuronal nuclei separated by fluorescence activated cell sorting (FACS). We also used cocaine self-administering mice, transcriptome sequencing, and *in vitro* studies to investigate the relationship between behavior, gene expression and methylation at this locus.

Results: We found cocaine-related hypermethylation, including within exon 8/9 of the *TH* gene. We were able to replicate this effect in an independent cohort (N=18 per group), and in the nucleus accumbens of chronically cocaine self-administering mice (N=8-10 per group). Methylation at this locus negatively correlates with *TH* expression in the CD of the cocaine group, and appears to be neuron-specific. Luciferase assays indicate that methylation of this region impedes enhancer activity.

Conclusions: Hypermethylation of *TH* is associated with chronic cocaine dependence and may have a regulatory role in this population. Our ongoing research uses epigenome editing techniques to understand the mechanisms through which methylation regulates *TH* transcription. Ultimately, work on this system will uncover the importance of epigenetic dysregulation of *TH* to the time course and trajectory of chronic cocaine dependence.

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Abstracts
CEEHRC Awardees

[70] Microbial imbalance is associated with a distinct pro-inflammatory epigenomic and transcriptomic profile of intestinal CD4 T cells from Inflammatory Bowel Disease patients

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Inflammation during Inflammatory Bowel Disease (IBD) is a complex, tightly regulated process initiated by tissue damage and caused by environmental factors that disrupt the microbiota composition and diversity. Failure to resolve the inflammatory process driven by the microbial imbalance leads to chronic inflammation, mainly induced and maintained by dysregulated CD4 T cell responses. Since it has become increasingly clear that epigenetic mechanisms play an important role in inflammation, we proposed to directly address the epigenetic regulation of CD4 T cell function during intestinal inflammation. Inflamed and adjacent non-inflamed biopsies were taken from IBD patients with the aim of identifying epigenetic changes that permit CD4 T cells to adopt pro- and anti-inflammatory features. By mapping the chromatin accessibility and gene expression of intestinal CD4 T cells, we found that 2,718 areas were more accessible in inflamed biopsies, whereas 665 areas were more common in non-inflamed biopsies. Pathways associated with T cell differentiation, regulation of transcription and regulation of T cell activation were associated with CD4 T cells from inflamed biopsies. In inflamed biopsies, motif analysis and expression of transcription factors (TFs) suggested an enrichment for binding sites of TFs known to induce and maintain Th1 (NFκB, STAT1, STAT4 and Tbet) and Th17 (RORA) cells. In addition, the binding affinity of these TFs was modulated by IBD-risk SNPs specifically in intestinal CD4 T cells from inflamed biopsies. As SNPs are germline and thus expressed in both inflamed and non-inflamed biopsies, we hypothesized that the microbiota was distinctly driving the activation of these TF in both sites of the gut. Indeed, the phylum proteobacteria was largely found in inflamed biopsies, which compromised the microbiota diversity and promoted a bacterial imbalance in the inflamed sites of the gut when compared to non-inflamed biopsies. To better characterize the CD4 T subpopulations in inflamed and non-inflamed biopsies, we performed single cell RNA-Seq. We discovered 7 different clusters of CD4 T cells in inflamed and non-inflamed biopsies. We found that the most prevalent cluster in inflamed biopsies shared characteristics of pro-inflammatory Th1 and Th17 lymphocytes, whereas the most predominant cluster in non-inflamed biopsies shared markers usually found in classical regulatory T cells. Our data indicate that the microbial imbalance in specific areas of the gut is associated with a distinct pro-inflammatory epigenomic and transcriptomic profile of intestinal CD4 T cells, which share functional similarities to Th1 and Th17 lymphocytes.

[115] Chromatin rearrangements observed over the earliest stages of hematopoiesis are co-opted to drive leukemogenesis in NPM1c Acute Myeloid Leukemia

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Chromatin changes during the first stages of hematopoiesis, from Hematopoietic stem cells (HSCs) to their nearest progeny Multipotent Progenitors (MPPs), are poorly understood. Minor differences at chromatin post-translational modifications and some gene expression changes have been identified, but don't explain the loss of self-renewal between these populations. Understanding the mechanisms driving these changes is important in finding cures to multiple hematological malignancies, such as Acute Myeloid Leukemia (AML). Survival rates for AML are poor (<50% for intermediate risk patients) and the presence of stem-like cells which can evade chemotherapy to drive tumour re-initiation is a major complicating factor.

We used ATAC-Seq to profile accessible chromatin in 96 AML fractions (sorted by CD34/CD38 markers from 24 bulk samples) classed as leukemia stem cells-positive (LSC+) or negative (LSC-) based on in-vivo engraftment. ATAC-seq data from these AML samples were compared to equivalent data from 13 sorted populations from human cord blood, including HSCs and MPPs.

We identified chromatin accessibility signatures over both hematopoiesis and AML, which revealed a signature that uniquely defined non-HSC progenitors. Genomic regions associated with this signature reveal a role for modulation of the chromatin looping factor CTCF. In-vitro assays on cord-blood derived progenitors showed CTCF is essential for quiescence exit and lineage commitment in HSCs but not MPPs, where CTCF-associated chromatin rearrangements have occurred.

In AML, a CTCF-enriched signature is observed in fractions with Nucleophosmin1 mutations (NPM1). Transfecting the NPM1c mutation into human cord blood induces changes to chromatin accessibility similar to the non-HSC progenitors, only in the most primitive cells. Together, our work suggests that NPM1c can reprogram the chromatin accessibility landscape in AML to hijack normal hematopoietic processes which regulate exit from quiescence.

Abstracts
Poster Presentations

[1] Inhibition of DNA-methylation enhances functional recovery only after removal of partial outlet obstruction but not during establishment of obstruction

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Introduction and Objective: Bladder remodelling due to partial bladder outlet obstruction (PBO) follows a sequence of overlapping processes of inflammation, hypertrophy, hyperplasia and fibrosis, and leads finally to entrenched bladder pathology. Epigenetic changes, such as DNA methylation, are believed to contribute to this persistent pathology despite adequate treatment of the outlet obstruction. We hypothesized, that a hypo-methylating agent such as decitibine would enhance recovery either during PBO or after removal of PBO.

Materials and Methods: 42 Sprague-Dawley female rats underwent PBO by tying a silk suture around both the proximal urethra and a 0.9mm steel rod. The rod was then removed, leaving the suture in place. Sham operation without tying the suture was performed in 24 rats. PBO and sham animals were randomized to 6-week obstruction (OB) or 6 week PBO plus de-obstruction (dOB). OB were further randomized to vehicle (normal saline) or DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (DAC, decitibine) at 1mg/kg three days/week intraperitoneally for 4 weeks. This dose had proven hypomethylating activity. Sleep cycle bladder voiding patterns were recorded at the 6 week timepoint for all animals. For the de-obstructions (dOB), the obstructing ligature was removed after PBO for 6 weeks and animals were treated for 4 weeks with DAC or vehicle. For dOB shams, after 6 weeks, we exposed the proximal urethra to control for the de-obstructing surgical procedure. Four weeks after the secondary procedure, we recorded sleep cycle bladder voiding patterns and residual volumes. Bladder and body weights, HT-QPCR and pyrosequencing of specific loci were analysed after OB at 6 weeks or dOB at 10 weeks. In vitro models of stretch and denatured matrix+/- DAC were explored to model the gene expression patterns uncovered by HT-QPCR.

Results: Treatment with DAC over 4 weeks **during** obstruction had a deleterious effect on function, whereas treatment with DAC after de-obstruction resulted in improved function. Among the dOB animals, DAC treatment helped preserve micturition volume with a significant increase of the micturition fraction (ratio of the mean voided volume/maximum bladder capacity) **by one third** ($p=0.05$). Gene expression analysis showed that the YAP/WWTR1 pathway was highly upregulated in OB bladders (CTGF, Cyr61, FN1). DAC treatment lead to 100- and 27-fold increases in BDNF and CTGF, above sham levels, respectively ($p<0.001$), alongside perturbation in function and decreased DNMT expression. During dOB, typical YAP/WWTR1 targets returned to lower levels. Interestingly, DAC increased in dOB BDNF consistent with improved function. In vitro and in vivo data supported a mechanistic role for DNA methylation in control of the YAP and WWTR1 pathway during obstruction and de-obstruction.
Conclusion: In bladders persistently altered by PBO, inhibition of DNA-methylation enhances functional recovery. This contrasts the effect of DNA methylation inhibition during partial obstruction, which exacerbates obstructive pathology and signaling. Further analysis into the epigenetic mechanisms of control of the WWTR1 and YAP pathways is underway.

[2] Identification of elements controlling variable escape from X-chromosome inactivation

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Background
X-chromosome inactivation (XCI) is the process by which one of the X chromosomes in XX females is silenced in order to express similar levels of X-linked genes with XY males. This silencing is not complete, as 12% of X-linked genes escape XCI in all females and an additional 15% of genes on the X are variable in whether they escape or are subject to XCI between females. Genes that escape from XCI have expression differences between males and females and are hypothesized to be a source of phenotypic variation. Variably escaping genes provide a unique opportunity to compare genes which are subject to or escaping from XCI, in the same genomic context. Variable escape from XCI may be due to underlying genetic differences as suggested by correlations within ethnic populations and between twins. Epigenetic differences may also lead to variable escape from XCI as tissue specific differences in escape are observed

Experimental Approaches & Results
To examine variably escaping genes, we use a panel of 18 female cell lines with extreme skewing of XCI resulting in the same X chromosome being inactive in all cells of the line. SNP pyrosequencing assays were designed for variable genes and used to measure the ratio of allelic expression in cell lines informative for those SNPs, allowing XCI status calls to be made for each gene in a subset of cell lines. Out of 17 genes examined currently, 13 have been shown to be both inactivated and escaping across different cell lines in our panel. A domain of multiple variably escaping genes was examined to determine if there was a domain level pattern behind variable escape genes but no discernible pattern was found and XCI status varied on a gene-by-gene basis. Methylation levels were examined using bisulfite converted DNA and pyrosequencing and compared to each gene's XCI status to determine any correlation between the two. Methylation levels tended to be in an intermediate range between the hypomethylation of escaping genes and hemi-methylation of inactivated genes, with limited correlation to XCI status.

Conclusions
Variable escape from XCI was seen in a panel of 18 female cell lines with skewed XCI. Genes within a domain differed in their XCI status on a gene by gene basis despite reports of topological associated domains impacting XCI status and correlating highly with which genes are constitutively escaping across all individuals. Chromosome Conformation Capture in our skewed cell lines is being undertaken to compare how chromosome conformation differs when XCI status differs for variably escaping genes. Methylation levels showed poor correlation to a gene's XCI status as expected when compared to genes which are constitutively subject to or escaping XCI. A bioinformatic analysis of larger datasets is being undertaken to determine if similar expression and methylation trends are seen. Understanding variable escape from XCI will further our understanding of XCI and epigenetic regulation as a whole.

[3] Epigenetic adaptation in the *Xlr* gene cluster following a transient loss of *Dnmt1* expression in mouse embryonic stem cells

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In early embryos, a major reprogramming wave resets genome-wide DNA methylation profiles. Throughout this wave, DNA methylation patterns, acquired in a parent-of-origin-manner on differentially methylated regions (DMRs) (i.e., imprinting genes), must escape this reprogramming and sustain precise DNA methylation profiles through continuous DNMT1 (DNA methyltransferase 1) activity. Using an embryonic stem (ES) cell model with inducible *Dnmt1* repression (*Dnmt1*$^{tet/tet}$), we recently showed that a temporary lack of *Dnmt1* triggers the permanent loss of DNA methylation profiles on various regions — designated as DMR-like regions— across the genome. We currently do not understand why DMR-like regions are unable to re-establish their DNA methylation profiles following the re-expression of *Dnmt1*, and what are the consequences on their expression profiles. Here we will focus on a particular subset of DMR-like genes; the X-linked lymphocyte-regulated (*Xlr*) family, as genes with potential chromatin remodeling functions in dendritic spine development and synaptogenesis. **Objective:** Define how a temporary lack of DNA methylation maintenance remodels the chromatin landscape associated with the *Xlr* cluster and determine how these changes modulate associated gene expression. **Results:** Following the temporary lack of DNMT1-dependant DNA methylation maintenance, the promoter regions of *Xlr3c*, *Xlr4a*, *Xlr4b* and *Xlr5c*, which were highly methylated (>75%), substantially and permanently lost DNA methylation. To determine if the loss of DNA methylation in the promoter regions of *Xlrs* alters the occupancy of histone modifications (H3K4me3, H3K27me3, and H3K27ac), we performed a series of ChIP-Seq assays in *Dnmt1*$^{tet/tet}$ ES cells prior, during and following the inactivation of *Dnmt1*. Our H3K4me3 (active mark) ChIP-Seq results revealed a sharp increase in peaks within the transcriptional start site (TSS) of *Xlr3c*, *Xlr4a* and *Xlr4b*. Similar observation were made for H3K27ac (active mark), in the TSS of *Xlr3c* and *Xlr5c*, whereas a substantial decrease was observed for H3K27me3 (inactive) in the TSS of *Xlr3c* and *Xlr4a*. Our RNA-Seq results confirmed that the overall remodeling of the chromatin landscape in the *Xlr* gene cluster lead to the strong activation of all family members. **Conclusion:** The present study highlights new perspectives on how alterations in DNMT1-dependent methylation maintenance can alter DNA methylation profiles, histone modification cross-talk and gene expression for DMR-like regions. We know that permanent loss of DNA methylation in *parent*-of-*origin* specific *DMR* leads to various imprinting defects linked with several human *disorders*. The role of DMR-like *Xlr* family members remain unclear, but our results suggest that like DMRs, lack of proper maintenance of DNA methylation profiles on these genes during early embryo development could negatively influence their future expression and lead to abnormal development. Further investigation is needed to determine the mechanism behind such epigenetic remodeling events following a temporary shortage of *Dnmt1* and if these permanent perturbations in epigenetic profiles can be overturned.

[4] Linking the chromosome architecture with transcriptional regulation during normal and disease development

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Accessing the genetic information is critical to establish the proper gene expression programs during normal and disease development. In addition to the chromatin environment, chromosomes twist, turn and bend in order to fit within the small nuclear space which increase complexity of the transcriptional process. We have shown that genes and noncoding regulatory regions are connected to create communities which provide the infrastructure for normal and aberrant transcriptional responses. At the center of the connections between the chromosome architecture and transcriptional regulation lies the mediator and cohesin complexes. Mutations in these components are associated with human diseases ranging from developmental syndromes to cancer. In the Cornelia De Lange Syndrome (CdLS), a complex developmental syndrome, mutations in the cohesin loader *NIPBL* and cohesin subunits are frequent. NIPBL and cohesin are important constituents of connected gene communities that are centrally positioned at noncoding regulatory elements. Accordingly, genes deregulated in CdLS are positioned within reach of NIPBL- and cohesin-occupied regions. Our findings suggest that connected gene communities provide a physical link to explain gene expression deregulation in human diseases. Therefore, we are moving our global understanding of gene regulation from single-gene to gene community-based approaches. Our most recent results confirmed communication between single noncoding regulatory regions, including promoter regions, and a community of genes. We are applying this conceptual framework to cancer problematics including the function of noncoding genetic events in addition to endocrine response and resistance.

[5] Does physical activity reduce the risk of breast Cancer by altering global DNA methylation? A review and meta-analysis of the evidence to Date

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Background: The aim of this review was to determine the extent to which the current literature supports the hypothesis that physical activity reduces breast cancer risk through changes in global DNA hypomethylation.

Methods: We conducted two systematic literature searches to identify epidemiologic studies which investigated the association between: 1) physical activity and global DNA methylation; or 2) global DNA methylation and breast cancer risk. These associations were quantified using DerSimonian and Laird random-effects models. Heterogeneity among studies was investigated through subgroup analyses and meta-regression, and was quantified using the Q-test and I^2 statistics.

Results: A total of 24 studies were included in this review. We observed a trend between higher levels of physical activity and higher levels of global DNA methylation (pooled SMD = 0.19; 95% CI: -0.03 to 0.40; p = 0.09) which, in turn, had a borderline significant association with a reduced breast cancer risk (pooled RR = 0.70; 95% CI: 0.49 to 1.02; p = 0.06). In subgroup analyses, a positive association between physical activity and global DNA methylation was observed among studies assessing physical activity over long periods of time (p = 0.02) and those measuring global DNA methylation in white blood cells (p = 0.02). Similarly, the association between global DNA methylation and breast cancer was statistically significant when excluding studies using the Luminometric Methylation Assay (p < 0.001).

Conclusions: Despite a heterogeneous evidence base, there is some suggestion that physical activity reduces the risk of breast cancer through reduced global DNA hypomethylation.

[6] New retroviral insertions drive widespread changes in the oocyte methylome

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Mouse oocytes display high levels of transcription originating from Long Terminal Repeats (LTR)-containing endogenous retroviruses (ERVs), with over 15% of all transcripts initiating in an LTR. The Mouse Transposon (MT) family of non-autonomous Mammalian apparent Long Terminal Repeats (LTR) retrotransposons (MaLRs) is the most highly active member, and in several cases, MT LTRs have been co-opted an oocyte-specific gene promoter. In the female germline, global *de novo* DNA methylation (DNAme) and establishment of gametic imprints takes place postnatally and is dependent on DNMT3A and transcription. Intersection with whole genome bisulfite sequencing (WGBS) suggests that transcription emanating from MT LTRs is responsible for transcription-coupled *de novo* DNAme at a significant fraction of genic and intergenic regions, many encompassing CpG island (CGI) promoters. Interestingly, MT elements are restricted to the rodent lineage, and, the highly active MTA elements are murine-specific.

To study the conservation of DNAme and transcription in mammalian oocytes we focused on mouse, rat and humans separated by 20 and 90 million years of evolution, respectively. We have identified hundreds of genes initiating in solo MT LTRs, several leading to species- or rodent-specific transcription bias. Human oocytes also exhibit high levels of MaLR transcription, particularly from the primate-specific THE-1 family, as well as from ERV1 LTRs, which are repressed in rodents. DNAme is highly correlated with transcription in each species, and we observe coding and non-coding transcripts initiating from LTRs which appear responsible for transcription-coupled *de novo* DNAme in a species-specific manner at a significant fraction of genomic regions, including CGI promoters. Taken together, these observations reveal that retrotransposons polymorphisms can have a profound effect on both the methylome and transcriptome in the mammalian germline.

[7] IHEC Data Portal 2017 update

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The IHEC Data Portal (http://epigenomesportal.ca/ihec) is the integrative online resource to navigate through datasets produced by the International Human Epigenome Consortium. As of October 2017, the Portal hosts over 10,000 human datasets, for which more than 8,000 are in the IHEC core set of assays. With an average of 250 unique sessions weekly, it is the central access point to visualize and obtain IHEC datasets. In order to increase information quality and accessibility, multiple new features have been added over the last year.

Such features include improved access to the Portal's underlying metadata, through search and filtering features, and using a more complete Web API that generates documents following IHEC Data Hubs specifications. Additional external tools over the UCSC Genome Browser, such as the Ensembl browser and GeEC, an external correlation tool embedded into a Galaxy instance, have been integrated. Moreover, as each consortium tracks are now locally hosted on the Portal's server, it is possible to generate IHEC tracks usage report, offering an overview of which IHEC resources are the most used by the epigenomics community. Other improvements include a more efficient correlation matrix tool, selected datasets metadata distribution in pie charts, enhanced session reports, and direct links to controlled access repositories to let users know where to go to request access to raw data.

Currently in the work is a method to allow the epigenomics community to share its generated data directly on the Portal, through the use of IHEC Data Hub documents, and offering different ways to compare this external data with IHEC data. A grid search feature based on any available metadata will also be released shortly. Details on these tools will be presented.

The IHEC Data Portal is a service hosted by GenAP (https://genap.ca), developed and maintained by the McGill Epigenomics Data Coordination Centre (http://epigenomesportal.ca). It is funded under the CEEHRC, by the CIHR and by Genome Quebec, with additional support from Genome Canada. The correlation matrix computation approach was developed by the Université de Sherbrooke, and funded by NSERC. The computing and networking infrastructure, and part of the software development, are provided by Compute Canada and CANARIE.

[8] Aberrant DNA methylation in childhood leukemia survivors and its correlation with neurocognitive late effects

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Advances in treatment of childhood acute lymphoblastic leukemia (ALL) have improved survival rates to nearly 95%. Treatment of ALL uses a combination of chemotherapy agents which include methotrexate (MTX). MTX inhibits dihydrofolate reductase (DHFR), an important protein in one-carbon metabolism (OCM), which generates methyl groups for a number of cellular processes including DNA methylation (DNAm).

Despite improvements in survival the majority of ALL survivors experience treatment-related late-term neurocognitive adverse outcomes. Our cohort of survivors completed a combination of neuropsychological and cognitive neuroscience measures which revealed deficiencies in working memory, processing speed as well as attention and behaviour issues. We hypothesized that ALL survivors, compared to neurotypical controls, have alterations in DNAm and that some of those changes will correlate with the observed neurocognitive late effects.

DNAm was assessed using the Illumina Infinium Methylation EPIC Beadchip array using blood samples from children that received chemotherapy for ALL (n=101) and had been in remission for 2 or more years. In a comparison between ALL survivors and neurotypical controls we have identified over 500 CpGs that were differentially methylated (FDR corrected $p<0.0001$). Enrichment analysis of these differentially methylated sites reveals a statistically significant over-representation of a number of GO molecular function categories including those related to histone methylation, growth and transcription factor binding (hypergeometric FDR q-value<0.001). Differential DNAm was also found when comparing ALL survivors with working memory and processing speed deficits to those in the cohort without such deficits.

This study has allowed us to begin to evaluate the impact of the treatment of ALL on the epigenome and its relationship to late-term neurocognitive outcomes. The identified DNAm alterations have the potential be used as biomarkers to identify ALL survivors at risk for adverse neurocognitive outcomes providing opportunities for early intervention.

[11] Design and use of a human sperm specific methylcapture-sequencing technique for the identification of environmentally susceptible loci in the human sperm methylome.

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How environmental exposures can affect human sperm have always been of great interest, as it can have consequences on future generations. DNA methylation is an epigenetic modification, occurring at approximately 30 million CpG dinucleotides across the genome. While reprogramming takes place following fertilization, some marks are maintained and may be transmitted to the embryo. Whole genome bisulfite sequencing (WGBS) can be utilized to examine methylation across the entire genome, but may be prohibitive due to its high costs, in particular, when analyzing large sample cohorts. Therefore, development of a targeted method would be an ideal approach for such large analyses. We used deep WGBS (average 23x coverage) on a pool of human sperm from a diverse group of donors (age, fertility status, *MTHFR* genotype and folic acid [FA] supplementation) in order to discover environmentally-susceptible sites of CpG methylation. Regions of particular interest (i.e. showing intermediate levels of methylation [20-80%]) were mainly located in intergenic and intronic regions, areas known to regulate gene activity. A targeted methylcapture-seq panel was designed to specifically target such regions, while also including content of the Illumina EPIC array. This initial capture panel examines ~830k regions that contain ~3.18 million CpGs distributed across the entire genome. As an initial test of this novel sperm-specific methylation panel, we re-sequenced human sperm from infertile patients given 5mg/day FA, previously analyzed by reduced representation bisulfite sequencing (RRBS). We observed min 1x coverage at an average of 11.98M CpGs, whereas targeted CpGs demonstrate intermediate methylation (46%) at much higher average coverage (28.5x) indicating efficient enrichment. Previous analysis of imprinted genes examined only a few CpGs (bisulfite pyro-sequencing) located within the imprinting control regions (ICRs) of imprinted genes. With our capture panel, DNA methylation across entire ICRs can be examined at single CpG resolution, and demonstrated little effect following FA supplementation. Similarly, our methylcapture-seq technique was able to recapitulate previous DNA methylation results by RRBS demonstrating that patients homozygous for the common polymorphism in *MTHFR* (TT), were affected to a greater extent than CC patients by FA. As well, while FA can act as methyl donor for reactions, supplementation was found to induce decreases in DNA methylation, in particular from TT subjects. Our sperm methylcapture-seq approach allows accurate assessment of DNA methylation profiles at single CpGs with an unprecedented coverage and in a cost-efficient manner. With this technology, we aim at identifying sperm DNA methylation signatures which could adversely be affected by environmental exposures and factors, such as folate, which in turn may be transmitted to the offspring and alter their development. (Supported by CIHR).

[12] Sex-specific DNA methylation changes in cord blood, maternal blood, and placenta from small for gestational age newborns in Bangladesh.

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Reduced linear growth in childhood is a major public health concern in the developing world. In Bangladesh 36% of children under the age of five (5.5 million) have moderate-to-severe linear growth reduction. A better understanding of early neonatal growth factors and their regulation could generate opportunities to ameliorate these outcomes. To study growth remediation strategies, 1300 pregnant mothers in Bangladesh were enrolled and infants were followed to two years of age. The high prevalence of growth restriction in this population is an ideal model in which to study its unexplored molecular mechanisms. In this cohort, more males (27%) than females (7%) had insufficient levels of IGF-1 in cord blood. Significantly more males (19%) than females (14%) were growth stunted at one year of age ($p<0.01$). Sex-specific epigenetic alteration of expression of growth-regulating genes may be causing the sex differences observed in this population. We hypothesized that sex-specific DNA methylation (DNAm) changes in cord blood are associated with small-for-gestational age (SGA) at birth.

We selected two groups from our participants for a DNAm pilot study: SGA infants (n=6 males & n=6 females) and average weight-for-age controls (n=6 males & n=6 females) for DNA methylation (DNAm) analysis. The average length-for-age z-score (LAZ) of the SGA boys (-2.17) and girls (-2.72) also differed significantly from male (0.19) and female (0.57) controls, meaning both birth weight and length were lower in the cases. We collected maternal blood, cord blood, and placenta (fetal and maternal sides). The Illumina Infinium Methylation EPIC array was used for DNAm analysis. Two between-group comparisons were made within each tissue to identify differentially methylated CpG sites: Male SGA vs male controls, female SGA vs female control.

In cord blood, the male SGA group had 950 differentially methylated sites, and SGA females had 1088 when compared to controls (Mann-Whitney U test, $p<0.05$, delta beta > 0.08). ~3 times as many differentially methylated sites were present in the placenta samples. The genes demonstrating DNAm changes fall into different functional categories in males vs. females, and across tissues. In female SGA placenta, DNAm changes were enriched at T-cell-related genes while in males, androgen and gonadotropin biosynthesis genes were enriched. Genes associated with height and timing of puberty were enriched in male and female SGA infants. There were sex-specific changes in genes encoding IGF-family proteins, including reduction in methylation at IGF binding protein-3 (*IGFBP3*) in male cord blood. In maternal blood, 203 probes distinguished male SGA births from controls, but a parallel signature could not be identified for females. These data show DNAm changes are present at growth-related genes in the cord blood of SGA infants, and that like the growth phenotypes that emerge in infancy, these changes are sex-specific.

[13] H3S10ph broadly marks early-replicating domains in interphase ESCs and shows reciprocal antagonism with H3K9me2

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Phosphorylation of histone H3 at serine 10 (H3S10ph) by Aurora kinases plays an important role in mitosis; however, H3S10ph has also been observed at regulatory regions of inducible genes in interphase mammalian cells, implicating mitosis-independent functions. Using the fluorescent ubiquitin-mediated cell cycle indicator (FUCCI) system, we found that 30% of the genome in mouse embryonic stem cells (ESCs) is marked with H3S10ph in interphase. H3S10ph broadly demarcates gene-rich regions in G1 and shows a strong positive correlation with domains of early DNA replication timing (RT) and negative correlation with H3K9me2 and lamin-associated domains (LADs). Consistent with mitosis-independent kinase activity, this pattern was preserved in ESCs treated with Hesperadin, a potent inhibitor of Aurora B/C kinases. Disruption of H3S10ph by expression of non-phosphorylatable H3.3S10A results in ectopic spreading of H3K9me2 into adjacent euchromatic regions, mimicking the phenotype observed in *Drosophila JIL-1* kinase mutants. Conversely, interphase H3S10ph domains expand in *Ehmt1*/*Glp*$^{-/-}$ ESCs, revealing that H3S10ph deposition is restricted by H3K9me2. Strikingly, spreading of H3S10ph at RT transition regions (TTRs) is accompanied by aberrant transcription initiation of genes co-oriented with the replication fork in *Glp*$^{-/-}$ and *Ehmt2/G9a*$^{-/-}$ ESCs, indicating that G9A/GLP may play a role in establishing repressive chromatin on the leading strand during DNA synthesis. H3S10ph is also anti-correlated with H3K9me2 in interphase murine embryonic fibroblasts (MEFs), and is restricted to intragenic regions of actively transcribing genes by G9A/H3K9me2. Taken together, these observations reveal that H3S10ph may play a general role in restricting the spreading of repressive chromatin in interphase mammalian cells.

[14] Modeling the interplay of epigenetic modifications leading to oncogenesis

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The identification of oncogenic mutations in histone genes - histone 3 p.K27M, p.G34V/R, and p.K36M - in various cancers[1,2] has brought forward novel targets in epigenetics for cancer therapy. However, the mechanisms of how these histone mutations cause cancers are still not clear. Researchers observed that the mutations inhibit histone modifying complexes: K27M inhibits PRC2[3] while K36M inhibits SETD2[2], leading to a global reduction of corresponding histone modifications, H3K27me3 and H3K36me3. Furthermore, the genome-wide distribution patterns of those modifications are also changed in disease tissues. It has been found that modifications at certain positions (e.g. K27 and K36) are antagonistic[4]. For example, reducing H3K36 methylation allows abnormal spread of H3K27me3, leading to excessive silencing of the genome. We postulate the interplay of histone modifications (as well as other epigenetic modifications such as DNA methylation) is crucial to understanding the downstream regulatory cascade leading to oncogenesis.

In this study, we aim to infer the interactions among epigenetic modifications in cancer cell lines, to elucidate the mechanisms of epigenetics-driven oncogenesis. Variations of epigenetic modifications have been shown to be associated with transcription dynamics and various diseases such as cancers. However, how the modifications interact and how the interactions lead to cancers are less studied. In addition, existing computational methods of modeling interactions of epigenetic modifications mostly focus on the signals in promoter regions[5,6,7], which may limit their power in identifying the regulatory interactions that determine cell fates. Here we will systematically explore the interactions of epigenetic modifications in different genomic regions to obtain a full picture of the interplay of modifications. Then by comparing the genome-wide interactions of modifications in normal cells and cancer cells, we are going to identify the critical interplay of epigenetic modifications underlying oncogenesis.

In conclusion, our study investigates the genome-wide interactions of epigenetic modifications and their relation to oncogenesis, which can not only help understand the downstream effects of perturbation to epigenetic modifications, but also predict the functions of epigenetic modifications in regulating cell fates.

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[15] Upregulation of miR-489-3p and miR-630 inhibits oxaliplatin uptake in renal cell carcinoma by targeting OCT2

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Renal cell carcinoma (RCC) is the most common urologic malignancy, accounting for 2%–3% of all adult malignancies. Approximately 70% to 85% of renal cell carcinoma is clear renal cell carcinoma. MiRNAs are a class of small non-coding RNAs (19~25nt), regulating their target genes expression at post-transcription level. The effect of traditional chemotherapy which is considered as a main regimen in the treatment of RCC is limited for drug resistance. The down-regulated expression of organic cation transporter OCT2 is a potential factor leading to oxaliplatin treatment resistance in RCC.

In this study, we observed that miR-489-3p and miR-630 could suppress OCT2 expression through directly binding to the OCT2 3'-UTR. Meanwhile via 786-O-OCT2-miRNAs stable expression cell models we found that miRNAs could repress the classic substrate MPP+, fluorogenic substrate ASP+ and oxaliplatin uptake by OCT2 both in vitro and in xenografts. In 33 clinical samples, miR-489-3p and miR-630 were significantly upregulated in RCC, negatively correlating with OCT2 expression level compared with adjacent normal tissue by tissue microarray analysis and qPCR validation. The increased binding of MYC to the promoter of pri-miR-630 responsible for the upregulation of miR-630 in RCC was further evidenced by chromatin immunoprecipitation and dual-luciferase reporter assay.

Taken together, this study indicated that miR-489-3p and miR-630 functioned as oncotherapy-obstructing microRNAs by directly targeting OCT2 in RCC. Combination of miRNA inhibitors in RCC therapy could sensitize cells to oxaliplatin and have important significance for guiding clinical chemotherapy.

[18] Utilizing Cell Line Models with Chromatin Remodelling Protein Deficiencies to Study Aggressive Dedifferentiated Endometrial Cancer

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An estimated 6600 Canadian women were diagnosed with uterine cancer in 2016, with 20% of women succumbing to the disease. One of the most lethal subsets of uterine cancer is dedifferentiated endometrial carcinoma (DDEC). DDEC tumors possess both well-differentiated and undifferentiated regions. The majority of metastatic disease is made up of cells from the undifferentiated component of DDEC yet it is unclear how these poorly differentiated regions are initiated and sustained within the carcinoma.

Examining the well-differentiated and undifferentiated components of DDEC tumors in terms of mutation profiles, we demonstrated that 80% of the undifferentiated regions in DDEC lesions lack the expression of core chromatin remodeling proteins, BRG1 or ARID1A and ARID1B. We hypothesize that the loss of these proteins, which are known regulators of transcription, may lead to the induction and/or maintenance of stem cell-like gene expression programs that drive dedifferentiation, metastasis and therapy resistance.

BRG1-deficient or ARID1A/B co-deficient cell line models were generated by CRISPR and validated using immunofluorescence and immunohistochemistry. qRT-PCR and immunofluorescence were used to assess the level of expression and localization of markers of epithelial-to-mesenchymal transition (EMT), stemness and endometrial lineage. The ability of the generated knockouts to proliferate and form spheres was evaluated, as well as an examination of the response of these BRG1-deficient or ARID1A/B co-deficient cell line models to current clinically relevant epigenetic inhibitors (vorinstat and tazemetostat). Tumor formation in immune-compromised mice will be monitored to ascertain any histological differences between wildtype and BRG1 or ARID1A/B knockout endometrial cancer cells.

BRG1 deficient endometrial cancer cell lines have been shown to partially undergo EMT and recapitulate the clinical DDEC phenotype. Determining the extent to which loss of BRG1 or ARID1A/B contributes to the acquisition of DDEC is a critical step towards improving diagnostic and treatment practices for aggressive stem-like forms of gynecological cancers.

[20] Repurposing Cardiac Glycosides to Reprogram Transcriptome and Acetylome in High C-MYC Expressing Cancers

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Cardiac glycosides are approved for heart failure treatment, but are also known to trigger anticancer and epigenetic effects. Our goal was to better define their epigenetic effects to identify cancer specific indications for their repurposing in the clinic. We demonstrated that low dose treatment with cardiac glycoside proscillaridin efficiently reprogrammed T-cell leukemia and rhabdomyosarcoma cells transcriptome towards a less proliferative and more differentiated phenotype through inhibition of cancer-specific super-enhancers such as C-MYC. Proscillaridin induced gene downregulation correlated with genome-wide loss of H3K9, H3K14, and H3K27 acetylation, which was caused by the loss of expression of the lysine acetyltransferases KAT3A, KAT3B, KAT5 and KAT2A. Proscillaridin also reduced lysine acetylation levels in non-histone proteins such as C-MYC, C-MYC protein partners, and chromatin remodelers. Furthermore, we demonstrated that proscillaridin is specifically effective against C-MYC driven cancer cells, which defines for the first time a molecular target for their repurposing. Therefore, we demonstrated that cardiac glycoside proscillaridin induces epigenetic reprogramming with specificity towards C-MYC driven cancers.

[21] Epigenetic Regulation of TAP-1 to Promote Immunogenicity of Tumor Cells

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Downregulation of the transporter associated with antigen processing 1 (TAP-1) has been observed in many tumors and is closely associated with tumor immunoevasion mechanisms, growth, and metastatic ability. The molecular mechanisms that underlie the relatively low level of transcription of the TAP-1 gene in cancer cells are largely unexplained. We previously discovered that epigenetic regulation plays a fundamental role in controlling tumor antigen processing and immune escape mechanisms. We found that the lack of TAP-1 transcription in TAP-deficient cells correlated with low levels of recruitment of the histone acetyltransferase (cap-binding protein or CBP) to the TAP-1 promoter. This resulted in lower levels of histone H3 acetylation at the TAP-1 promoter, which subsequently lead to decrease in accessibility of the RNA polymerase II complex to the TAP-1 promoter. These observations suggest that CBP-mediated histone H3 acetylation normally relaxes the chromatin structure around the TAP-1 promoter region, allowing transcription. In addition, we found a hitherto-unknown mechanism wherein interferon gamma upregulates TAP-1 expression by increasing histone H3 acetylation at the TAP-1 promoter locus. In further studies, we found the histone deacetylase inhibitor (HDACi) Trichostatin A (TSA) was also able to increase TAP-1 expression, resulting in an increase in immunogenicity of the tumor cells and an overall *in vivo* anti-tumoral effect. These findings lie at the heart of understanding immune escape mechanisms in tumors and suggest that the reversal of epigenetic codes may provide novel immunotherapeutic paradigms for intervention in cancer. We are now setting up a screen to look for molecules that target the histone acetylation to determine whether we can improve TAP-1 expression in tumors with small molecules.

[22] Molecular architecture of the essential yeast histone acetyltransferase complex NuA4 redefines its multi-modularity

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A key mechanism for regulating gene expression involves post translational modifications (PTM) of nucleosomal histones, which can lead to changes in chromatin structure or can act as signaling events that regulate the association of factors critical for transcription regulation. Acetylation, the covalent linkage of an acetyl group to the epsilon amine of lysine, is a major histone PTM correlated with active transcription. Conserved from yeast to humans, the NuA4 histone acetyltransferase is a large multisubunit complex essential for cell viability through regulation of gene expression, genome maintenance, metabolism, and cell fate during development and stress. How the different NuA4 subunits work in concert with one another to perform these diverse functions remains unclear, and addressing this central question requires a comprehensive understanding of NuA4's molecular architecture and subunit organization. Here, we report the structure of fully assembled native yeast NuA4 determined by single-particle electron microscopy. Our data revealed that NuA4 adopts a trilobal overall architecture, with each of the three lobes constituted by one or two functional modules. By performing crosslinking coupled to mass spectrometry analysis and *in vitro* protein interaction studies, we further mapped novel intermolecular interfaces within NuA4. Finally, we combined these new data with other known structural information of NuA4 subunits and subassemblies to construct a multi-scale model to illustrate how the different NuA4 subunits and modules are spatially arranged. This model shows that the multiple chromatin reader domains are clustered together around the catalytic core, suggesting that NuA4's multi-modular architecture enables it to engage in multivalent interactions with its nucleosome substrate.

[23] Histone Arginine Methylation and Transcriptionally Active Chromatin

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Chicken has long been recognized as a model system to study the organization and function of a vertebrate genome. Its genome is almost three times smaller than the human genome, but has about the same number of genes, with 60% of them having a single human ortholog. Moreover, there are long blocks of conserved synteny between the chicken and human genomes. In terms of chromosomal organization of genes, the human genome is closer to the chicken than to rodents. Additionally, following 310 million years of separate evolution, conserved noncoding sequences are likely to highlight functional elements in both chicken and human genomes. We recently reported the genome-wide profiles of chromatin signatures (H3K4me3, H3K27ac, salt-soluble domains) in relation to expression levels in chicken polychromatic erythrocytes (PMID: 27226810). In the current study, we will show the genomic distribution of the histone modifying enzymes protein arginine methyltransferases 1 and 5 (PRMT1, PRMT5) and their products H4R3me2a (asymmetric) and H3R2me2s (symmetric), respectively. Further we mapped the open nucleosome-depleted regions using FAIRE-sequencing. We will present our novel findings which show the genomic distribution of the PRMT enzymes and their substrates in relation to other active histone marks and the organization of the transcriptionally active gene domains in the chicken polychromatic erythrocyte. This work was supported by funds from NSERC and a Canada Research Chair to J Davie.

[24] A Preclinical Mouse Model for H3.3K27M Paediatric High-Grade Gliomas

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Primary brain tumours are a leading cause of cancer-related mortality in children less than 15 years old. In particular, paediatric high-grade gliomas (pHGG) are associated with 20% OS at 5 years. Studies from our group and others have led to the identification of recurrent somatic, lysine-to-methionine mutations in residue 27 of histone H3.3 (H3.3K27M) in paediatric high-grade gliomas (pHGG). H3.3K27M has been shown to exert a dominant-negative effect on H3K27 trimethylation (H3K27me3) via sequestration of PRC2, a protein complex implicated in such key biological processes as cellular differentiation, development, and stem-cell plasticity. Despite these advances into the pathology of the disease, efforts to investigate its preclinical development as well as the identity of the cell-of-origin continue to be deterred by the lack of faithful preclinical model for H3.3K27M pHGG.

To address these issues, we describe a mouse model of H3.3K27M HGG, which faithfully recapitulates human H3.3K27M pHGG. We report that H3.3K27M and loss of p53 are sufficient for neoplastic transformation during a specific window of brain development, and that concomitant gain of wild-type Pdgfra decreases the latency of tumourigenesis. Next, we characterize the effect of H3.3K27M on the transcriptome. To show that the transcriptomic changes observed in the mouse model are generalizable to human H3.3K27M pHGG, we assembled a data set of human tumours with matching molecular alterations. A cross-species comparison of the H3.3K27M signatures reveals that the mouse model recapitulates human tumours from the transcriptomic standpoint. We then characterize the impact of the mutation on the epigenome, as well as how it relates to changes in the transcriptome. Our analyses show that although there is a significant loss of H3K27me3 genome-wide, loss in genic regions is variable. However, loss of the mark is associated with a general upregulation of the underlying genes, consistent with the notion of epigenetic de-repression. To demonstrate that the changes in H3K27me3 levels too are consistent with human tumours, we compared H3K27me3 deposition patterns in the mouse model to those in human H3.3K27M pHGG and found a high level of agreement among orthologs, particularly for genes under the regulatory control of PRC2. Finally, we show that loss of H3K27me3 events of *de novo* deposition of H3K27me3, although rare, can still occur in spite of a global decrease in the mark. Overall, this *in vivo* model will shed light on our understanding of the pathology and progression of pHGG, highlight windows of opportunity during disease progression for treatment, as well as provide a reliable preclinical platform for drug testing.

[25] Concentration-response changes in global genome epigenetic markers induced by “data-poor” copper and zinc organometallics in short-term *in vitro* assays using the HC04 human liver non-cancer cell line.

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Cu- and Zn-organometallics have multiple applications (pesticides, fungicides, dietary supplements, personal care products, wood preservatives, dyes, paint, pigments), and many are considered “data-poor” because the available toxicological information is insufficient to assess their health risks. There is a growing awareness that global changes in DNA methylation and histone post-translational modifications (PTM) contribute to metal toxicity and cancer development. Previously, we demonstrated that copper-dimethyldithiocarbamate (CDMDTC) was the most toxic among a series of nine "data-poor" chemicals. Here, in order to inform about the specific chemical structure responsible for inducing toxicity, effects of CDMDTC were compared to those induced by Cu or Zn and to the non-metal components (dimethyldithiocarbamate: DMDTC). Effects on cell proliferation, on global genome changes in DNA methylation, and on histone-3 PTM (H3K9ac, H3pan-ac, H3K27me3, H3K4me3, H3K9me3) were measured in a non-cancer human liver cell line (HC-04) by flow cytometry and a histone multiplex assay on a Luminex system. Cu alone, and DMDTC alone, had little effects when tested independently, however, they were found to synergize when tested in combination. CDMDTC induced DNA damage and epigenetic disruption at 0.2 µM and was the most potent “data-poor” chemical. CDMDTC was found to induce small but statistically significant changes in DNA methylation with a clear concentration-response H3 hypoacetylation. Hypoacetylation can be a consequence of changes in pH, DNA damage, energy metabolism or enzymatic deregulation. Modifications to the histone protein, reduction in DNA methylation, and induction of DNA damage, are all intermediary steps toward abnormal cellular transformation, which raises concerns that altering these mechanisms CDMDTC might induce cancer predisposition. The ability of CDMDTC at inducing cell transformation, a type of pre-cancer lesion, will be investigated. Overall, this project provides concentration-response curves that are essential to the risk assessment process, it informs on chemical structure-activity relationships, and provide data for “data-poor” chemical prioritization for risk assessment and for the investigation of their potential to cause cancer predisposition.

[27] Ethical, Legal and Social Implications (ELSI) of Epigenetics: Preliminary Findings of a Systematic Literature Review

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Recent years have seen an increasing number of academic publications discussing the potential ethical, legal and social implications (ELSI) of epigenetics. To better understand the nature, origins and scope of discussions, we conducted a systematic review of this emerging literature. Two investigators independently searched for English or French peer-reviewed articles, commentaries and academic book chapters, published from January 2005 to December 2016, and discussing the ELSIs of epigenetics. Variations and permutations of keywords such as 'epigenetics', 'DNA methylation', 'ethical', 'legal', 'social', 'issue', 'implication', 'concern', 'justice', 'responsibility', 'discrimination', 'privacy' and 'confidentiality', have been used for the search. The reference tables of identified papers were then scrutinized for missing publications, allowing the inclusion of publications by scholars from a large diversity of fields of study. After reading more than half (>60) of the total number of publications (n=108) selected, the two investigators agreed on the prevalence of nine (9) general themes, or sensitive "areas of discussion", with regards to the potential implications of epigenetics for society. All publications were then independently coded for: area of discussion, journal type (discipline), general tone of the paper (i.e., neutral, optimistic or cautionary), and style of argumentation (i.e., descriptive, empirical, rhetorical, dialectical or reflexive). The two coding tables were subsequently compared and merged into an integrated version. In cases of first stance disagreement, we openly discussed our individual analyses and reasoning, and simultaneously scrutinized the paper one more time to reach agreement. In this talk, I will present and discuss the preliminary findings of our systematic review of the potential ELSIs of epigenetics.

[28] Differential Susceptibility to Early Life Socioeconomic Stress in Children's Genome-wide DNA Methylation

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Child socioeconomic status is a key predictor of child health outcomes, theoretically with socioeconomic status acting as a proxy for child stress exposure. The responsiveness of epigenetics to environmental exposures could allow epigenetics to function as a mediator of the demonstrated connection between child socioeconomic status and child health outcomes. The connection between child stress exposures, epigenetics and health outcomes are most commonly explored through DNA methylation epigenome wide association studies, with promising results. In addition to the associations seen between DNA methylation and child exposures, observations of many DNA methylation quantitative trait loci in the human methylome suggest allelic variants can have strong effects on DNA methylation levels. The nexus between child exposures, DNA methylation and allelic variants is a promising space to explain a further component of variability in DNA methylation.

Here we have examined the interaction between childhood socioeconomic status, DNA methylation and allelic variants using 178 children's buccal epithelial cell DNA at age 9 years from the Peers and Wellness Study (PAWS). The samples have data from the Illumina infinium 450K for DNA methylation and the Psychiatric Genomics Consortium PsychChip array for genotyping. CpG and SNPs were examined if a pair was within 5kb and linear models were used with an interaction term between genotype and family income per dependent. We found 11,812 CpGs with nominally significant interactions ($p<0.01$; delta beta > 0.05), between genotype and family income. As the samples available with the necessary deep-phenotyping, genetic and epigenetic information are limited, we did not have a large enough sample size to perform stringent multiple test corrections. We did, however, have the opportunity to independently validate the observed interactions. Of our observed interactions 39 CpG-SNP pairs validated ($p<0.01$; delta beta > 0.05) in 290 independent samples from the Gene Expression in Kids Only Collective (GECKO).

The interactions observed between DNA methylation, allelic variants and family income suggest a differential susceptibility to income level exposure where sensitivity is specified by the genotype. This model of differential susceptibility of DNA methylation to childhood stress would need follow-up mechanistic testing at individual loci to define the nature of the association between DNA methylation and genotype in response to stress, in the context of a complex biological network. However, the potential explanatory effect of differential susceptibility in social epigenetics could begin to explain the complexity and often unexplained variability seen in DNA methylation.

[29] Genome wide meta-analysis of parent-of-origin effects of asthma and related phenotypes in four cohorts

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Background: Asthma is a complex disease caused by a combination of genetic and environmental factors. The main genetic effects of the common SNPs identified by Genome-Wide Association Studies (GWAS) do not fully explain the heritability of asthma. Genetic effects related to parent-of-origin, such as genomic imprinting, may help us better understand the etiology of asthma and related phenotypes. Imprinting is an epigenetic phenomenon where the expression of genes depends on their parental origin.
Hypothesis: We hypothesized that genomic imprinting (parent-of-origin effects) is involved in the etiology of asthma and related phenotypes (atopy and airway hyperresponsiveness (AHR)).
Methods: To identify candidate genomic regions for imprinting we used GWAS data from four family-based studies (trios). These studies are: 1) the Canadian Asthma Primary Prevention Study (CAPPS), 2) the Study of Asthma Genes and Environment (SAGE), 3) the Saguenay–Lac-Saint-Jean Québec Familial Collection (SLSJ), and 4) the Dutch Asthma GWAS (DAG). We used a likelihood-based variant of the Transmission Disequilibrium Test. Parent-of-origin effects were analyzed by including parental sex as a modifier in the analysis, which determines whether the asthma risk is modified by the parental origin of the allele. Meta-analyses were conducted using the results of SLSJ, DAG (available only for asthma), and the joint analysis of CAPPS and SAGE, weighted by the number of informative transmissions for each study.
Results: For asthma, the number of SNPs that showed significant ($P \leq 1.49\times10^{-5}$) parent-of-origin effects (maternal/paternal) in each cohort was as follows: 1 SNP in SLSJ (251 trios); 4 SNPs in DAG (316 trios); 7 SNPs in the joint analysis of CAPPS and SAGE (141 trios). Of particular interest, the joint analysis of CAPPS and SAGE identified a parent-of-origin effect at a known imprinted gene, *CTNNA3*. This gene has previously been associated with occupational asthma in a GWAS study. Of the significant results, 8 out of 12 of the SNPs were in or near long non-coding (lnc)RNA genes. LncRNAs are known to be involved in genomic imprinting and gene regulation. Meta-analysis for asthma, using results of SLSJ (251 trios), DAG (316 trios), and the joint analysis of CAPPS/SAGE (141 trios), resulted in 5 SNPs with significant parent-of-origin effects with $P \leq 1.49\times10^{-5}$. All these five SNPs were in or near lncRNA genes. Meta-analysis for atopy, using results of SLSJ (229 trios) and the joint analysis of CAPPS/SAGE (217 trios), yielded 6 SNPs, 5 of which were located in or near lncRNA genes. The meta-analysis for AHR on SLSJ (132 trios) and CAPPS/SAGE (219 trios) resulted in 8 SNPs with significant parent-of-origin effects, 7 of which were located in or near lncRNA genes.
Conclusion: Meta-analysis for asthma yielded 5 SNPs with significant POE ($P \leq 1.49\times10^{-5}$) in or near lncRNA genes. We will conduct further analyses based on multinomial modeling and haplotype estimation (using EMIM software) to confirm our results.

[30] Role of JMJD2A/KDM4A lysine demethylase in heterochromatin maintenance during oncogene-induced senescence

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Cellular senescence is characterized by a permanent arrest of proliferation occurring in response to activated oncogenes, DNA damage, oxidative stress or shortened telomeres. Senescence is an important tumor suppression process, but also a possible contributor to tissue aging *in vivo*. In human cells, chromatin structure is extensively remodeled upon senescence entry, as evidenced by accumulation of HP1 proteins, H3K9me2/3 and histone variant macroH2A in specialized domains of dense chromatin, called Senescence-Associated Heterochromatin Foci (SAHF). These structures contribute to the stabilization of the senescence phenotype by silencing proliferation-promoting genes. Furthermore, H3K9me3 contribute to deposition of H4K20me3 at pericentric heterochromatin, and this mark has been recently shown to accumulate during senescence. Here we propose to study the interplay between H3K9me3 and H4K20me3 during oncogene-induced senescence.

Methods and Results:
Methylation on H3K9 has been shown to be regulated by members of the Jumonji family including JMJD1A-B and JMJD2A-D (KDM4A-D). KDM4A also binds H4K20me3, through its hybrid tandem Tudor domain. Since H4K20me3 is frequently lost in human cancer, whereas KDM4A is frequently overexpressed, we analyzed the relationship between H4K20me3, H3K9me3, SAHF formation upon KDM4A expression, in the context of oncogene-induced senescence (OIS). Our results suggest that deposition of H3K9me3 and H4K20me3 in the SAHF is inter-dependent. We demonstrated that KDM4A expression blocks the formation of heterochromatic foci and that KDM4A catalytic activity is required to disrupt SAHF formation. KDM4A ectopic expression contributes to senescence reversal, thus suggesting a potential mechanism by which elevated expression of KDM4A might promote transformation.

Conclusion and Relevance:
Our results strongly suggest that histone lysine methylation regulate the stability of the senescence response to oncogenes and expose the potential of senescence induction by targeted inhibition of KDM4A in the treatment of cancer.

[31] Blood triglyceride levels are associated with DNA methylation at the serine metabolism gene *PHGDH*

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Efficient interventions to reduce blood triglycerides are few; newer and more tolerable intervention targets are needed. Understanding the molecular mechanisms underlying blood triglyceride levels variation is key to identifying new therapies.

To explore the role of epigenetic mechanisms on triglyceride levels, a blood methylome scan was conducted in 199 individuals from 5 French-Canadian families ascertained on venous thromboembolism, and findings were replicated in 324 French unrelated patients with venous thromboembolism. Genetic context and functional relevance were investigated.

Two DNA methylation sites associated with triglyceride levels were identified. The first one, located in the *ABCG1* gene, was recently reported, whereas the second one, located in the promoter of the *PHGDH* gene, is novel. The *PHGDH* methylation site, cg14476101, was found to be associated with variation in triglyceride levels in a threshold manner: cg14476101 was inversely associated with triglyceride levels only when triglyceride levels were above 1.12 mmol/L (discovery *P*-value = 8.4×10^{-6}; replication *P*-value = 0.0091). Public databases findings supported a functional role of cg14476101 on *PHGDH* expression. *PHGDH* catalyses the first step in the serine biosynthesis pathway.

These findings highlight the role of epigenetic regulation of the *PHGDH* gene in triglyceride metabolism, providing novel insights on putative intervention targets.

[32] Rare Variants in PRC2 Complex Members: Genotype-Phenotype Correlations

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Patients who have clinical features of the rare growth disorder called Weaver syndrome are frequently found to have pathogenic coding variants in *EZH2*. Pathogenic mutations in *EZH2* have been proven to cause tall stature, macrocephaly, skeletal abnormalities such as scoliosis and dysmorphic facial features, and intellectual disability.

Recently, patients with phenotypes similar to Weaver Syndrome who test negative for *EZH2* mutations have been shown to have pathogenic coding variants in other members of the PRC2 complex, namely *EED* and *SUZ12*. We will present data on mutation-positive patients in our cohort, including rarer patients with growth failure instead of overgrowth, and one patient with normal intelligence and an unusual myopathy. We will also discuss ongoing efforts to assess the specific effects of these mutations on the activity of the PRC2 complex, and to model the effects of these mutations in non-human organisms.

[33] An environment-dependent transcriptional network specifies human microglia identity.

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Introduction and Objective
Microglia are the tissue-resident macrophages of the brain and play key roles in its development, homeostasis, and protection against diseases. As innate immune cells, microglia play a critical role in the inflammatory response associated with neurodegenerative diseases. In spite of the important roles of microglia in these diseases, little is known about the transcriptional mechanisms underlying their functions in the human brain. To address this problem, we employed paradigms and techniques in epigenetics and next-generation sequencing to precisely characterize the genomic regulatory elements landscape and gene expression profile of human microglia.

Methodology
Microglia were isolated from human brain samples resected from pediatric patients undergoing neurosurgeries to treat epilepsy or brain tumors. Tumor samples consisted of incidentally/pathology-negative resected tissue, and not the tumor itself. Brain samples underwent mechanical homogenization within 3 hours following resection, and live-$CD11b^{+}CD45^{Low}CD64^{+}CX3CR1^{+}$microglia were purified with flow cytometry. Isolated microglia were then assayed for gene expression by RNA-seq and epigenomic activity by ChIP-seq (e.g. H3K4me2, H3K27ac).

Results
Gene expression profiles as assessed by RNA-seq showed great consistency across patients. Importantly, human microglia express high levels of *CX3CR1*, *P2RY12*, and *TMEM119*, which is coherent with mouse microglia. ChIP-seq analyses revealed that distal genomic regulatory (i.e. enhancers) in human microglia display strong enrichment for DNA motifs recognized by PU.1, IRF, MEF, and RUNX transcription factors. The gene expression profile and enhancer repertoire of human microglia both require constant inputs from signals present in the brain. Indeed, isolated microglia maintained in a tissue culture environment for seven days display profound changes in gene expression. This is associated with a loss of PU.1-IRF, IRF/ISRE, MEF2, and SMAD epigenomic activity. Finally, our data enables to expand the annotation of numerous DNA variants associated with multiple sclerosis. Of note, six of these variants previously not annotated localize within PU.1-bound genomic regulatory regions in microglia.

Conclusions
Overall, our work identifies key signaling pathways that regulate gene expression in human microglia and provides an important resource for the interpretation of DNA variants that may predispose for the development of neurodegenerative diseases, including MS.

[34] Investigating how mutations in the CMG helicase cause Primordial Dwarfism

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Genetic and epigenetic information is transmitted across cell divisions largely in the replicative S-phase, and accumulating evidence indicates that mutations in genes involved in DNA replication are associated with multiple diseases. Mutations in *CDC45*, *MCM5*, and *GINS1* genes, members of the CMG helicase complex, have been associated with specific types of Primordial Dwarfism (PD) - a group of rare genetic disorders characterized by profound pre- and post-natal growth defects and reduced life expectancy. CMG (CDC45/MCM2-7/GINS1-4) is the evolutionarily conserved DNA helicase that unwinds DNA at the replication fork. Interestingly, we recently identified four individuals presenting a novel type of PD characterized by mutations in the DNA replication factor-encoding *GINS3* gene, another member of the CMG helicase complex. CMG DNA helicase activity is absolutely necessary for DNA replication, and our preliminary data shows that PD patient cells harboring mutations in the GINS3 protein accumulate in the replicative S-phase of the cell cycle.

We hypothesize that the identified GINS3 mutations negatively impact the CMG helicase stability leading to defects in DNA replication and cell proliferation that manifest in primordial dwarfism. To test this hypothesis, we are studying the impact of PD mutations on the CMG complex interaction with protein partners and *in vitro* helicase activity on naked DNA and on chromatin. To this end, we have generated cultured cell models to determine how CMG DNA helicase interacts with other proteins at replication forks. We are utilizing a proximity-dependent labeling BioID technique to characterize the WT and mutant GINS3 interactome. This will allow us to identify how WT and mutant CMG DNA helicases differ in their protein-protein interactions and hence in their functions at replication forks. Furthermore, we are examining the impact of these mutations on the stability of the CMG DNA helicase and its ability to unwind DNA in our *in vitro* helicase assay. Additionally, we will also investigate the impact of the PD mutations on CMG helicase activity over nucleosomes that are assembled on forked DNA templates, thus mimicking chromatin templates at the replication fork.

This study will thus identify the mechanism by which mutations in GINS3 protein affect DNA replication and cell proliferation and provide important insights into the biology of the CMG helicase activity.

[35] Roles of the imprinted gene *Mest* in embryonic development

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Background

Genomic imprinting is an epigenetic phenomenon by which gene expression follows a parent-of-origin-specific pattern. *Mest* (mesoderm specific transcript) is regulated by genomic imprinting and is paternally expressed in placental mammals. The *Mest* locus is located on proximal mouse chromosome 6, sharing syntenic homology with human chromosome 7q32, and contains the only differentially methylated CpG island in the region. *Mest* knockout (KO) mice, generated by gene targeting on the 129S1 strain background, are associated with embryonic growth retardation with no catch-up growth, and abnormal maternal behavior only upon paternal transmission of the knockout allele. Loss of MEST function may therefore contribute to the mUPD7 (maternal uniparental disomy for Chr7) phenotype associated with Sliver-Russell syndrome and characterized by growth retardation in humans.

The function of the MEST protein is unknown, but it belongs to the α/β-hydrolase fold family. Recent studies have shown that MEST may act as an inhibitor of the Wnt pathway, suggesting it is a positive regulator of growth, consistent with the roles of other paternally expressed genes. *Mest* is highly expressed in embryonic and extraembryonic mesoderm during development, including in the fetal endothelial cells of the labyrinth, a layer of the mouse placenta responsible for nutrient delivery to the embryo.

Experimental approach and results

To address the function of *Mest* in embryonic growth, we will utilize our *Mest* KO mutant mouse line previously generated. The KO line has been brought from the 129S1 to C57BL/6J reference strain background for further molecular studies. We have recapitulated the growth retardation phenotype seen *in utero* from E11.5 to E17.5. *Mest* KO embryos are approximately 20% smaller than their WT littermates. Placental weights at these time points are not significant between genotypes, suggesting that there may be structural abnormalities. RNA *in situ* hybridization experiments using placental markers, histology, and morphometric analyses show proper initiation of labyrinth formation at E11.5 to E13.5. However, starting at E15.5, KO placenta exhibit disorganization in the basement membrane of the labyrinth, and abnormal glycogen accumulation.

Conclusion

Defects in placental architecture may contribute to *in utero* growth retardation in *Mest* KO mice. RNA-seq on whole placenta is being undertaken to identify potential *Mest* targets. However, the lack of catch-up growth postnatally in *Mest* KO mice suggest *Mest* has a larger role in development. An *in vitro* reporter assay will be developed to test the role of *Mest* in regulation of the Wnt pathway. Imprinted genes have a fundamental importance in many biological processes. Our work will both provide insight into the function of *Mest* in developing tissues, and epigenetic regulation in early development.

[36] The Role of RXR in the Regulation of Myogenic Differentiation

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Epigenetic modification readout has provided us with plentiful information in order to thoroughly understand the regulation of genes during myogenic differentiation. While skeletal myogenesis is coordinated by sequential expression of myogenic regulatory factors including MyoD and myogenin, chromatin modifications have emerged as vital mechanisms of myogenic regulation. We have recently found that bexarotene, a clinically approved agonist of retinoid X receptor, promotes the specification and differentiation of muscle lineage; however, the genome wide impact of rexinoid action on myogenic expression has not yet been investigated. Through integrated RNA-seq and ChIP-seq analyses, we here found that bexarotene promotes myoblast differentiation through the coordination of exit from the cell cycle and the activation of muscle-related genes. We uncovered rexinoid-responsive residue-specific histone acetylation at a distinct chromatin state associated with MyoD and myogenin. We also define a new mechanism of rexinoid action which is mediated by the receptor and largely reconciled through a direct regulation of MyoD expression. Thus, we provide novel molecular insights into the interplay between retinoid X receptor signaling and chromatin states associated with myogenic programs in early differentiation.

[38] Epigenetic landscapes reveal transcription factors in the CpG island methylator phenotype in colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer in the world arising in the epithelial tissue of the colon or rectum with a majority presenting as adenocarcinomas. CRC is currently classified into four major subtypes (CMS1-4) based on genomic and transcriptomic features. CMS1, associated with genetic hypermutability, microsatellite instability and $BRAF^{V600E}$, is characterized by aberrantly methylated CpG islands (CGI) - a poorly understood phenomenon found in a wide range of tumour types known as CpG island methylator phenotype (CIMP). To further our understanding of epigenetic dysfunction in CRC we generated reference epigenomic profiles (ihec.org) of epithelial cells obtained from the distant (>50 mm) normal and tumor resections of six grade I CRC patients. From the 6 tumor samples profiled, four were found to be hypermutated. Promoter-associated CGIs in hypermutated samples were hypermethylated compared to both matched normal and hypomutated tumour samples. Pairwise comparisons of active enhancer states (H3K27ac/H3K4me1) between CRC samples and matched normal tissues revealed an overall increase in active enhancer states in CRC genomes compared to normal ($p=0.078$, median: 2.7% and 1.9% of genome, respectively). The common CRC specific active enhancer states and up-regulated H3K27ac peaks were enriched in cancer and adenocarcinoma related terms (Binomial FDR<0.01) and were hypomethylated in the CIMP associated CRC compared with non-CIMP CRC (Welch t-test $p=0.06$ and $p=0.04$, respectively for active enhancer states and H3K27ac peaks). We identified over-represented PU.1-IRF and ETV1 motifs in the up-regulated common and specific H3K27ac peaks in CRC. Integration of genome-wide H3K27ac modification and gene expression of the tumor samples showed enrichment of HOXA1, HOXC11, HMGA1, MSX2, ELK4, FOXD1, HNF1B, PAX6, TWIST2, ZNF35, and ZNF75D transcription factors (TFs) in the CIMP associated CRC samples. In summary, our analysis has revealed enhancer activity linked to CIMP tumorigenesis and identified key TFs associated with CIMP CRC tumors.

[39] Mapping chromatin accessibility changes in a *Xenopus* model of Fetal Alcohol Syndrome

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Fetal Alcohol Spectrum Disorder (FASD) is the most common cause of neurodevelopmental impairments in the western world, with an estimated prevalence of 2-5% in Canada. In addition to alcohol exposure during pregnancy (prenatal alcohol exposure, PAE), genetic and epigenetic influences are thought to play an important role in FASD outcomes. To study the kinetics of epigenetic changes induced by alcohol, we implemented the ATAC-seq method using our *Xenopus* embryo FASD model. *Xenopus* embryos were treated with ethanol, and samples were collected during early gastrula (the most sensitive stage to alcohol), late gastrula and late neurula. In parallel, embryos were treated with 4-diethylaminobenzaldehyde (DEAB) to inhibit retinoic acid biosynthesis, and untreated controls were collected. Each sample was processed for ATAC-seq library preparation and in parallel RNA was extracted for RNA-seq analysis. The ATAC-seq protocol centers on identifying accessible chromatin sites which extensibly overlap with DNaseI hypersensitive sites. These sites should mainly include gene regulatory elements accessible to transcriptional regulatory factors. Changes in the ATAC-seq signature will identify sites affected as a result of the ethanol or DEAB treatments.

To characterize the chromatin domains identified by ATAC-seq we took advantage of published data of other epigenetic marks at the same developmental stages. The regions identified by ATAC-seq in our control samples extensively overlap with regions identified by ChIP-seq for p300 and H3K4me3, both markers of gene regulatory elements actively involved in transcription. Peak distribution is enriched at promoter (20%) and 5'UTR (5%) regions, and in most cases, the corresponding genes are known to be expressed at embryonic day 10.5. Furthermore, peak density corresponds to the transcriptional start site (TSS) and show the TSS are enriched for nucleosome-free fragments and show phased nucleosomes similar to those seen by MNase-seq at the −2, −1, +1, +2, +3 and +4 positions.

Further data mining of ATAC-seq reveals new biological information about changes in gene regulation at this sensitive developmental time point. ATAC-seq peaks map to master Transcription Factors (TF) during gastrula and it will be interesting to identify new transcriptional targets that are supported by RNA-seq and by the presence of TF binding sites in target genes and candidate enhancers. Mapping chromatin accessibility changes will identify new transcriptional networks in development and in response to PAE. The primary goal of this project is the development of epigenetic assessment tools that can objectively assess the effects of alcohol during embryogenesis, and the risk of FASD. Identifying inexpensive and reliable biomarker(s) indicating PAE exposure effects and associated FASD outcomes will lead to more accurate and earlier identification of individuals at greatest risk, and support early intervention.

[40] Modeling methyl-sensitive transcription factor motifs with an expanded epigenetic alphabet

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Many transcription factors (TFs) initiate transcription only in specific sequence contexts, providing the means for sequence specificity of transcriptional control. A four-letter DNA alphabet only partially describes the possible diversity of nucleobases a TF might encounter. Cytosine is often present in the modified forms: 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC). TFs have been shown to distinguish unmodified from modified bases. Modification-sensitive TFs provide an additional epigenetic mechanism to modulate gene regulation and downstream expression. Mutations in DNMTs, TET2, or IDH1/2 often result in dysregulation of 5mC and 5hmC in both blood and brain cancers. Modeling TF binding to modified DNA, in both normal and cancerous cells, helps identify the epigenetic processes responsible for aberrant regulatory programs.

To understand the effect of modified nucleobases on gene regulation, we developed methods to discover motifs and identify TF binding sites in DNA with covalent modifications. Our models expand the standard A/C/G/T alphabet, adding m (5mC) and h (5hmC). We adapted the position weight matrix (PWM) formulation of TF binding affinity to this expanded alphabet. We engineered several tools to work with expanded-alphabet sequence and PWMs. First, we developed a program, Cytomod, to create a modified sequence, using data from bisulfite and oxidative bisulfite sequencing experiments. Second, new versions of MEME, DREME, and MEME-ChIP enable *de novo* discovery of modification-sensitive motifs. A new version of CentriMo enables central motif enrichment analysis to infer direct DNA binding in an expanded-alphabet context. We also added support for our alphabet to the RSAT matrix-clustering software, enabling clustering of modified PWMs. These versions permit users to specify new alphabets, anticipating future alphabet expansions.

We created expanded-alphabet genomes using whole-genome maps of 5mC and 5hmC in naive *ex vivo* mouse T cells and 5mC in K562 leukemia cells. Using this sequence and ChIP-seq data, we identified modification-sensitive *cis*-regulatory modules. We elucidated known methylation binding preferences, in both cell types, including C/EBPβ's preference for methylated motifs and c-Myc's preference for unmodified E-box motifs. We then discovered novel preferences for 5 TFs, as well as numerous new 5mC and 5hmC motifs. We have begun *in vivo* tests of several predictions, planning to conduct ChIP-BS-seq and CUT&RUN. Computationally inferring transcription factor modification sensitivities facilitates experimental investigation of dysregulated oncogenic pathways.

[41] Identification of a specific PRC2 associated lncRNA and its role in somatic cells adaptation under stress

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Long non-coding RNA (lncRNA) and chromatin associated multi-protein complexes interactions are known as vital aspects of epigenetic regulation. Polycomb Proteins (PcG) – Discovered in *Drosophila melanogaster* – as chromatin proteins that contribute to the process of “transcriptional memory” are prototypes of this regulatory pathway. PcG proteins form two main complexes: Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2). More than 1000 long non-coding RNAs (lncRNA) have been shown to associate with PRC2. However, their specificity and their functional role remain to be elucidated. Previous studies were mainly focused on the PcG-lncRNA interactions in Embryonic Stem Cells (ESCs). In this study, we investigated the crosstalk between lncRNA and PcG proteins, and their function in somatic cells, particularly mouse skeletal muscle cells (C2C12), to understand their regulatory role in adaptation to stress. The project relies on a novel mechanism of PRC2 activation in response to stress allowing in vivo genome wide global assembly and activity of this complex at their target sites (Bodega et al., NSMB, 2017). Interestingly, in this Study we could find a specific lncRNA that might be essential in recruitment of PRC2 during Stress of somatic cells.

[42] Gene expression profiling reveals abundant tissue and sex-specific changes across pubertal development

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The timing of human puberty is highly variable, sexually dimorphic, and associated with adverse health outcomes. Over 20 genes carrying rare mutations have been identified in known pubertal disorders, many of which encode critical components of the hypothalamic-pituitary-gonadal (HPG) axis. Recent genome-wide association studies (GWAS) have identified more than 200 candidate genes at loci associated with age at menarche or voice breaking in males. We know little about the spatial, temporal or postnatal expression patterns of the majority of these puberty-associated genes.

Using a high-throughput and sensitive microfluidic quantitative PCR strategy, we profiled the gene expression patterns of the mouse orthologs of more than 175 puberty-associated genes in male and female mouse HPG axis tissues, the pineal gland, and the liver at five postnatal ages spanning the pubertal transition. The most dynamic gene expression changes were observed prior to puberty in all tissues. We detected known and novel tissue-enhanced gene expression patterns, confirming the sensitivity of our method.

Notably, over 40 puberty-associated genes in the pituitary gland showed sex-biased gene expression, most of which occurred peri-puberty. These sex-biased genes included the orthologs of candidate genes at GWAS loci that show sex-discordant effects on pubertal timing. To further explore the sex-dimorphism in pituitary gene expression, we profiled the whole transcriptome of all pituitary samples using RNA-seq and built co-expression modules of sex-biased genes. Overall, our findings provide new insights into the expression of puberty-associated genes and support the possibility that the pituitary plays a role in determining sex differences in the timing of puberty.

[43] Circular RNA regulates expression of drug metabolism enzymes in hepatocellular carcinoma

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Circular RNA (circRNA) is an abundant class of noncoding RNA, characterized by covalently closed loop structures with neither 5' to 3' polarity nor polyadenylated tails. Because it isn't easily digested by ribonuclease, it is highly stable in vivo compared with their linear counterparts. According to circulation mechanisms, there are about three kinds of circRNA, such as ecircRNA (exonic circular RNA), ciRNA (intronic circular RNA) and ElciRNA (exon- intron circular RNA). CircHIPK3 formed by direct back-splicing of Exon2 of the HIPK3 gene due to the long intronic complementary repeat elements. It is predominantly in the cytoplasm and regulates cell growth by binding to multiple miRNA, including a well-known tumor suppressor miR-124.

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death world-wide, especially in China. Paclitaxel is a chemotherapy medication used to treat a number of types of cancer. Owing to the lower efficiency of a single agent, the combined effects of Sorafenib (a first-line drug for HCC) and paclitaxel have always been explored. Paclitaxel is a classic substrate of cytochrome p450(CYP)2C8. CircRNA may regulate the expression of CYP2C8 to influence the effective concentration of paclitaxel.

CircHIPK3 highly expressed in the Hepatic carcinoma cell lines by qRT-PCR detection. Because circRNA can be targeted by small interference RNA, we used RNA interference to silence the expression of circHIPK3. The results showed that silencing of circHIPK3 decreased the expression of CYP2C8. Meanwhile, expression plasmid with circRNA sequence and the two inverted repeat elements was constructed and transfected into HCC cells. The expected circRNA production was detected by qRT–PCR analysis. Overexpression of circHIPK3 increased the expression of CYP2C8.In collusions, circHIPK3 can regulate the expression of CYP2C8. CircHIPK3 is significantly highly expressed in HCC compared with normal tissue. High expression of CYP2C8 may reduce the concentration of paclitaxel by metabolism. Therefore, detection of circRNA expression has an important significance for guiding clinical chemotherapy drugs.

[45] Molecular characterization of the role of RUNX1 in Notch signalling in T-cell Acute Lymphoblastic Leukemia (T-ALL)

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T-cell Acute Lymphoblastic Leukemia (T-ALL) subgroups are characterized by arrest at different stages of thymocyte development and aberrant expression of transcription factors. NOTCH1 signalling is critical for T cell fate specification and development. NOTCH1 undergoes oncogenic activation in >50% of T-ALL cases. RUNX1 is a transcription factor that works downstream of NOTCH1 during normal T cell development. However, overexpression of RUNX1 blocks normal maturation of T cells and leads to accumulation of premature leukemic blast cells. In *Drosophila* NOTCH1 loads onto chromatin after modification of enhancers in haemocytes by a homolog of RUNX1, Lozenge, a so-called "pioneer factor". In human T-ALL, RUNX1 is associated with NOTCH1 regulated enhancers but the molecular mechanism of RUNX1 to cooperate NOTCH1 is not clearly understood in hematologic malignancies.

To study the role of NOTCH1 and RUNX1 in T-ALL, we knocked down NOTCH1 by pharmacologic inhibition and RUNX1 by lentiviral shRNAs. In order to dissect the molecular mechanism(s) underlying these phenotypes, we have performed RNA-seq and ChIP-seq against a panel of histone modifications (H3K4me1, H3K4me3, H3K27ac, H3K27me3) for samples that have either been depleted of RUNX1 or NOTCH1.

We find that human T-ALL patient-derived samples and cell lines are sensitive to both RUNX1 depletion by lentiviral shRNAs and pharmacologic Notch pathway inhibition. Our results suggest that RUNX1 plays central role in global maintenance of H3K27ac by both direct and indirect interactions with other transcription factors and coactivators. RUNX1 dependent enhancer states drive the expression of proto-oncogenes associated with leukemic pathways. RUNX1-KD leads to the blockage of G1-S phase of cell cycle by deregulating cell cycle dependent kinases. NOTCH1 and RUNX1 interaction and/or colocalization is the key event to maintain H3K27ac levels by simultaneous eviction of H3K27me3 by NOTCH1 and recruitment of H3K27ac by RUNX1. At the RUNX1+NOTCH1 binding sites, there is a significant loss of active marks (H3K4me3 and H3K27ac) upon RUNX1-KD and gain of repressive mark (H3K27me3) in NOTCH1-KD samples. RUNX1 alone or NOTCH1 alone did not activate some of the key NOTCH1 target genes, suggesting cooperative action of RUNX1 and NOTCH1. Examples of such NOTCH1 targets include MYC, DTX1, IGF1R etc.

The functional synergy of RUNX1 and NOTCH1 may form a multimeric complex to recruit components of the transcriptional machinery and/or acetylate chromatin, results in an increase in H3K27ac level and decrease of H3K27me3 that opens the chromatin to be accessible by the other members of the NOTCH1 complex.

[46] High resolution 3D genome architecture of glioblastoma

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Recent technological advances have highlighted the pivotal role of 3D genome architecture in the etiology of pathological states, including cancer. It has been shown that aberrant looping of DNA allows spurious promoter-enhancer interactions and causes gene dysregulation in the absence of obvious genetic lesions. Hypothesizing that erroneous DNA looping is an important part of glioblastoma biology, we generated high resolution Hi-C contact maps of three patient-derived primary glioblastoma cultures. We sequenced 2.6 billion paired-end reads with 1.4 billion valid read pairs remaining after filtering and deduplication to define the 3D genome architecture at sub-5-kb resolution for each sample. To the best of our knowledge, this is the highest resolution Hi-C dataset available for primary cultures from cancer patients. Examination of contact maps at 250-kb resolution has revealed genetic anomalies including inversions, reciprocal translocations, and double minute chromosomes. Analysis at 50-kb resolution has revealed broad regions of A/B compartment switching between patient samples, with corresponding changes in contact frequency between neighboring topologically-associated domains. Finally, at 5-kb resolution we identified precise sites of DNA looping including the CTCF binding motifs anchoring the loop, thus defining potential hotspots for SNVs or small indels. Altogether, this work thoroughly details patient-specific differences in 3D genome architecture, allowing for identification of novel epigenetic regulatory sites and future targets for precision medicine.

[49] TOP2A and EZH2 provide early detection of an aggressive prostate cancer subgroup

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Background: Current clinical parameters do not stratify indolent from aggressive prostate cancer (PCa). Aggressive PCa, defined by the progression from localized disease to metastasis, is responsible for the majority of PCa-associated mortality. Recent gene expression profiling has proven successful in predicting the outcome of PCa patients, however they have yet to provide targeted therapy approaches that could inhibit a patient's progression to metastatic disease.
Methods: We have interrogated a total of seven primary PCa cohorts (N = 1,900), two metastatic castration resistant PCa datasets (N = 293) and one prospective cohort (N = 1,385) to assess the impact of *TOP2A* and *EZH2* expression on PCa cellular program and patient outcomes. We also performed immunohistochemical staining for TOP2A and EZH2 in a cohort of primary PCa patients (N = 89) with known outcome. Finally, we explored the therapeutic potential of a combination therapy targeting both TOP2A and EZH2 using novel PCa-derived murine cell lines.
Results: We demonstrate by genome-wide analysis of independent primary and metastatic PCa datasets that concurrent TOP2A and EZH2 mRNA and protein up-regulation selected for a subgroup of primary and metastatic patients with more aggressive disease and notable overlap of genes involved in mitotic regulation. Importantly, TOP2A and EZH2 in PCa cells act as key driving oncogenes, a fact highlighted by sensitivity to combination-targeted therapy.
Conclusions: Overall, our data supports further assessment of TOP2A and EZH2 as biomarkers for early identification of patients with increased metastatic potential that may benefit from adjuvant or neo-adjuvant targeted therapy approaches.

[51] Genome-wide analysis of neonatal naïve CD4 T cells

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Sepsis kills about one million infants each year in the world, and is the number one cause of mortality under 28 days of age. Many of these deaths are vaccine preventable. Yet, most vaccines are designed based on our understanding of the adult immune system, and are thus not very effective in the newborn period. To understand how the neonatal immune system functionally differ from the adult immune system, we conducted the first side-by-side genome-wide methylome and transcriptome comparison of neonatal and adult naïve CD4 T cells, which are essential in order to develop a long-term immunological memory to re-infection. Unexpectedly, we found extensive differential methylation and gene expression age differences encompassing 4.5% of CpG sites, and 26% of all detectably expressed genes. A gene set enrichment and hallmark pathway analyses indicated important differences in Th17 differentiation, transforming growth factor beta (TGF-β) signaling and IL-7-driven homeostatic expansion pathways. Functionally, neonatal CD4 T cells were unable to produce the mucosal-targeting cytokine IL-17 when antigenically stimulated in Th17-polarizing conditions. Rather, these cells produced high levels of IL-22, which may be more age-adapted to the newborn period.

In summary, we demonstrate broad age-related molecular difference in human CD4 T cells. Moreover, our data highlight fundamental aspects of the biology of neonatal CD4 T cells, involving a large network of epigenetic and gene expression events. This knowledge may eventually help design vaccines that are more effective in newborns. An immediate implication of our data is that systems level analyses of human T cells (and likely also of other hematopoietic cell lineages) absolutely need to take into account the origin of these cells (i.e. whether they were obtained from adult peripheral blood or cord blood) when interpreting data, and contrary to several example studies in the literature. Studies are underway to understand how epigenetics regulate these age-specific gene expression events through modulation of the chromatin/histone structure.

[52] Histone Acetylation, Not Stoichiometry, Regulates Linker Histone Binding in *Saccharomyces cerevisiae*

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Linker histones play a fundamental role in shaping chromatin structure, but how their interaction with chromatin is regulated is not well understood. In this study, we used a combination of genetic and genomic approaches to explore the regulation of linker histone binding in the yeast, *Saccharomyces cerevisiae*. We found that increased expression of Hho1, the yeast linker histone, resulted in a severe growth defect, despite only subtle changes in chromatin structure. Further, this growth defect was rescued by mutations that increase histone acetylation. Consistent with this, genome-wide analysis of linker histone occupancy revealed an inverse correlation with histone tail acetylation in both yeast and mouse embryonic stem cells. Collectively these results suggest that histone acetylation negatively regulates linker histone binding in *S. cerevisiae* and other organisms.

[53] Hypoxia: epigenetic modulator in the induction of cancer cell plasticity

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Phenotypic plasticity affords cancer cells with the ability to metastasize and resist therapies, leading to reduced survival in patients. A tumor micro-environmental factor that can lead to an induction of plasticity is hypoxia, a condition of low oxygen. Tumor hypoxia can lead to an induction of various stem cell and epithelial-to-mesenchymal transition markers, surrogates for measuring cancer cell plasticity. Hypoxic reprogramming of the cancer cell can be achieved through changes in the epigenome, with global alterations in histone modifications.

Here we propose to address how the epigenomes of breast cancer cells respond to hypoxia by examining the alterations in histone modifications in concert with the resulting transcriptional response. T47D and MDA-MB-231 breast cancer cell lines, epithelial and mesenchymal-like respectively, were used. Using ChIP-seq, we examined the global changes in repressive (H3K27me3) and active (H3K4me3) histone marks, which were chosen for their association with hypoxia and regulation of the stem cell phenotype. Structural changes in chromatin was interrogated using electron spectroscopic imaging. RNA-seq was employed for characterization of the resulting transcriptome.

We identified striking differences in the hypoxic responses of T47D and MBA-MB-231 cells, encompassing epigenetic and transcriptional alterations. T47D cells responded more robustly to chronic hypoxia (0.5% O_2, 48 hours), in both histone modification alterations, overall chromatin condensation, and differentially expressed genes. Differentially expressed genes in T47D cells were enriched in hypoxia response, epithelial-to-mesenchymal transition, and metabolism gene sets. Additionally, hypoxia induced genes in T47D were characterized by concordant increase in H3K4me3 marks just upstream of the TSS and a decrease in H3K27me3 marks both upstream and downstream of the TSS. Taken together, our findings indicate that the epigenetic and transcriptional responses to hypoxia are highly context dependent and heterogeneous and further suggests that epigenetic alterations may contribute to transcriptomic profiles of breast cancer cells. Elucidating the nuanced cellular response to hypoxia and the role of the epigenetic alterations in this response will better our understanding of hypoxia induced cancer cell plasticity.

[54] Critical Roles of the Histone Methyltransferase MLL4/KMT2D in Metabolic Syndrome

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The pathophysiologic continuum of non-alcoholic fatty liver disease begins with steatosis. Despite recent advances in our understanding of the gene regulatory program directing steatosis, how it is orchestrated at the chromatin level is unclear. PPARγ2 is a hepatic steatotic transcription factor induced by overnutrition. Here, we report that the histone H3 lysine 4 methyltransferase MLL4/KMT2D directs overnutrition-induced murine steatosis via its coactivator function for PPARγ2. We demonstrate that overnutrition facilitates the recruitment of MLL4 to steatotic target genes of PPARγ2 and their transactivation via H3 lysine 4 methylation because PPARγ2 phosphorylated by overnutrition-activated ABL1 kinase shows enhanced interaction with MLL4. We further show that Pparg2 (encoding PPARγ2) is also a hepatic target gene of ABL1-PPARγ2-MLL4. Consistently, inhibition of ABL1 improves the fatty liver condition of mice with overnutrition by suppressing the pro-steatotic action of MLL4. Our results uncover a murine hepatic steatosis regulatory axis consisting of ABL1-PPARγ2-MLL4, which may serve as a target of anti-steatosis drug development.

[55] Endogenous retroviruses and the evolution of species-specific imprinted genes.

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Imprinted genes represent a group of ~100 genes that are monoallelically expressed in a parent-of-origin-specific manner in mammals, with paternally (*Pegs*) and maternally expressed genes (*Megs*) almost equally represented. Several of these genes are required for normal development and are implicated in diverse human syndromes and cancer. Since both active and silent alleles of these genes are present in the same nucleus, they are each regulated by different epigenetic marks. Most imprinted expression stems from DNA methylation (DNAme) marks or imprints, differentially inherited from the gametes and maintained throughout development as differentially methylated regions (DMRs). In the mouse, most DNAme imprints are of maternal origin and are established postnatally in growing oocytes, in a global wave of *de novo* DNAme leading to methylation of ~40% of the oocyte genome. RNA profiling (RNA-seq) and DNAme analysis by whole-genome bisulfite sequencing (WGBS) have shown that *de novo* DNAme occurs within transcribed regions in oocytes. Indeed, most maternal imprints associated with *Pegs* mark their CpG island (CGI) promoter which acquire DNAme because they are covered by an oocyte-specific transcript.

Previous work has shown that specific families of long terminal repeat (LTR) endogenous retroviruses are highly transcribed during oogenesis and consequently are responsible for a significant portion of the DNAme seen in mature oocytes. To understand the mechanism responsible for the evolution of new imprinted genes, we have analyzed genes imprinted in the mouse but not in human. We observed that for some of these species-specific imprinted genes, *de novo* DNAme at their CGI promoter appears to be a consequence of transcription initiating within an upstream retroelement, acting as an alternative oocyte-specific promoter. Using the CRISPR-Cas9 system, we have generated mutant mouse lines carrying precise deletions of the retrotransposons acting as alternative oocyte-specific promoters upstream of two paternally expressed imprinted genes, *Slc38a4* and *Impact*. Our analysis of heterozygous embryos with paternally or maternally inherited deletion alleles shows that imprinting is lost at both loci when the deletion comes from the oocyte. Our results suggest that species-specific imprinted genes can emerge during evolution via the insertion of transcriptionally active transposable elements.

[56] Early Embryonic Alcohol Exposure Induces DNA Methylation Dysregulation in Mid-Gestation Mouse Forebrain and Placenta

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Prenatal alcohol exposure (PAE) is known to altered epigenetic profiles in cells during brain development and be part of the molecular basis underpinning Fetal Alcohol Spectrum Disorders (FASD) etiology. However, the consequences of a PAE during very early embryonic life on the future epigenetic landscape of embryonic and extraembryonic tissues remain unknown. **Our research hypothesis is that a PAE during preimplantation will initiate DNA methylation dysregulation that will later be detectable in the developing conceptus**. We believe that these original epigenetic alterations will be perpetuated and amplified in the developing brain as well as in the placental tissue. To test this, we instigated FASD in mouse 8-cell embryos by injecting ethanol at 2.5 days of pregnancy (E2.5). We collected FASD (ethanol) and control (saline) E10.5 embryos and placentas. We then established genome-wide quantitative DNA methylation profiles of forebrains and placentas by Reduced Representation Bisulfite Sequencing (RRBS). Bioinformatic analyses of FASD (n=12) vs controls (n=8) samples revealed 686 and 2942 differentially methylated tiles (DMTs) in forebrain and placenta samples respectively. We also investigated the effect of alcohol exposition on gene expression by qPCR. Out of 14 genes tested —related to development, neuronal activities or epigenetic modifications— in E10.5 forebrain samples, 10 genes showed significant reduction of expression following ethanol-exposure at E2.5. Those genes include *Dnmt1*, *Ehmt1* and *Dclk2*. Unexpectedly, we highlighted sex-specific DNA methylation and gene expression perturbations in response to ethanol exposure. Interestingly, we also uncovered 21 specific regions abnormally methylated in both FASD forebrain and placenta samples. Our study establishes for the first time that early embryonic PAE can cause epigenetic dysregulations that leads to permanent alteration in the future epigenetic program of brain and placenta cells. The epigenetic dysregulations observed in FASD placental tissues allow us to believe that this tissue could be use for FASD risk assessment at birth. Altogether, our results allow us to have a better understanding of how early embryonic epigenetic perturbations can alter the normal function of the brain and lead to neurodevelopmental disorders present in children with FASD.

[57] Epigenetic Regulation by APOBEC3B in Cancer

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Cancer cells differ from their normal counterparts by genetic mutations, as well as by epigenetic changes in DNA methylation and chromatin accessibility profiles. The APOBEC3B cytosine deaminase is overexpressed in a large variety of tumors, including breast, lung, ovarian and head/neck cancers. APOBEC3B has been shown to mutate the cancer genome via its deamination activity, however it is unknown whether it additionally affects the epigenetic profile of these cells. Our biochemical studies show that APOBEC3B can deaminate methylated cytosines, making it a good candidate to act as a modifier of DNA methylation in cancer cells where APOBEC3B is overexpressed. Using CRISPR-Cas9, we created isogenic APOBEC3B-expressing and null breast cancer cells. These cells are being utilized for reduced representation bisulfite sequencing (RRBS) screens to identify regions in the genome which have APOBEC3B dependent methylation patterns. Our initial results indicate that APOBEC3B indeed functions as an epigenetic modifier of DNA methylation.

[58] CTCF haploinsufficiency in the developing brain results in an ASD and schizophrenia-like phenotype

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Transient defects during brain development can lead to disorders including autism and schizophrenia. Additionally, the regulation of higher order chromatin structure is now recognized as key to achieve normal neurodevelopment. CTCF is a transcription factor which regulates gene expression by modulating three-dimensional genome architecture, and mutations in CTCF have been identified in intellectual disability syndromes and schizophrenia. To study the role of CTCF in brain development we generated CTCF heterozygous mice which exhibit a ~30% reduction in CTCF in the developing brain during mid to late gestation in mice, but have recovered CTCF to near normal levels in adult mice. The CTCF heterozygous mice are viable and fertile, but exhibit hind-limb clasping, a behaviour indicative of neurological defects. A panel of behavioural tests showed that adult CTCF heterozygous mice are hyperactive, and that male but not female mice have decreased anxiety and altered social and aggressive behavior. Magnetic resonance imaging revealed altered brain morphology in CTCF heterozygous mice: a substantial reduction in the size of the anterior commissure connecting the two temporal lobes of the cerebral hemispheres, and in deep cerebellar nuclei. These behaviors and morphological brain defects resemble schizophrenia in humans. Current studies are using RNA-sequencing to identify transcriptional changes in the embryonic brain of CTCF heterozygous mice, to correlate these changes with CTCF binding sites identified by ChIP- sequencing, and to investigate how early gene expression changes could lead to the phenotypes seen in adults.

[59] Widespread hypermethylation of enhancers drives transcriptional deregulation in IDH1 mutant glioma

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Neomorphic mutations in the isocitrate dehydrogenases 1 gene (IDH1) are early and highly recurrent events in glioma. Mutant IDH1 (mIDH1) is thought to drive epigenomic dysfunction through the inhibition of α-ketoglutarate-dependent dioxygenases, including the TET family of DNA demethylases. To understand the role of mIDH1 in tumorigenesis, we profiled the epigenomes and transcriptomes of mIDH1 and IDH1 gliomas, as well as neural progenitor cells (NPCs) as a normal counterpart. Compared to NPCs, mIDH1 gliomas showed an expected global increase in DNA methylation enriched in CpG islands and promoters. Surprisingly, among the hypermethylated promoters associated with differentially expressed genes between mIDH1 and NPCs, 54% were up-regulated. To explore the mechanism of this unexpected finding, we integrated whole genome DNA methylation measurements with enhancer elements. We observed hypermethylation of promoter associated enhancers in glioma with a significant increase in mIDH1 harbouring gliomas. These hypermethylated enhancers were enriched in DNA elements recognized by distinct transcription factors in mIDH1 and IDH1 gliomas and included transcription factors that have been previously reported with increased binding affinities in presence of mCpG, such as NKX2.1. Furthermore, we found that vitamin C treatment increased hydroxymethylation level in mIDH1 glioma cell lines. In addition, vitamin C treatment significantly reversed the abnormal enhancer landscape and recruitment of transcription factors induced by mIDH1, leading to partial recovery from the oncogenic transcriptional program. Taken together our data suggests a novel mechanism of mIDH1 induced hypermethylation of promoter associated enhancers driving increased transcription factor recruitment and an oncogenic transcriptional program, and such effects can be potentially reversed by vitamin C treatment.

[61] Paternal folate deficiency increases birth defect severity in a transgenic mouse model representative of a vulnerable population

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Children in vulnerable populations are at an increased risk of developing complex diseases and exhibit a higher rate of birth defects. A father's environmental exposure to toxicants and poor diet influence disease transmission across generations, potentially through epigenetic inheritance. However, the mechanisms involved in such paternal transmission remain unknown. This study aims to elucidate the effects of an environmental stressor on offspring development using a mouse model that represents a vulnerable population due to their pre-existing abnormal sperm epigenome. Our transgenic mouse model overexpresses the lysine-specific histone demethylase KDM1A in developing sperm, resulting in an altered sperm epigenome such as may be found in men of vulnerable populations due to toxicant exposure, stress or poor nutrition. Adult sires with an altered sperm epigenome, or control mice with a normal sperm epigenome, were fed a folate deficient (FD) or folate sufficient (FS) diet and bred to control females on an FS diet. A quantitative skeletal analysis on embryonic day 18.5 mice revealed a significant increase in severe abnormalities in offspring sired by FD transgenics. To assess metabolic alterations, the transcriptome of fetal livers was evaluated by array to detect differential gene expression across experimental groups. We also performed a ChIP-sequencing experiment on the sperm of the adult sires, targeting the H3K4me3 mark, to identify changes associated with diet. This will be the first study to determine the role of the paternal sperm epigenome and environmental effects that may explain the health disparities that exist in vulnerable populations.

[62] Elucidating the function of neomorphic IDH mutations in Acute Myeloid Leukemia

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Introduction: Neomorphic isocitrate dehydrogenase (IDH) mutations are recurrent genetic lesions observed in Acute Myeloid Leukemia (AML) that drive accumulation of the metabolite D-2-hydroxyglutarate (D2-HG). D2-HG is an inhibitor of 2-oxoglutarate-dependent dioxygenases (2-OGDD), a family of enzymes that include a class of histone and DNA demethylases. To investigate the role of D2-HG in primary FLT3ITD AML blast cells we performed native ChIP-seq in primary AML cells harboring FLT3ITD in the presence and absence of neomorphic IDH mutations.

Methods: Chromatin immunoprecipitation sequencing (ChIP-seq) targeting histone3 lysine 4 tri-methylation (H3K4me3), H3K4me1, H3K27me3, H3K27ac, H3K36me3, and H3K9me3 was conducted on aliquots of 10,000 bone marrow and cord blood derived CD34+ cells and primary AML blast cells harboring the FLT3ITD allele and IDH2R140Q , IDH2R172H, IDH1R132H, or wild type alleles. The resulting data were integrated with existing matched RNA-seq and whole genome bisulfite sequencing datasets.

Results: Comparisons of the H3K27me3 landscape across normal CD34+ cells, AML-IDH2WT-FLT3ITD and AML-IDHMu-FLT3ITD revealed a unique H3K27me3 profile in AML-IDHMu-FLT3ITD cells. Matched RNA-seq datasets confirmed transcriptional repression of genes with H3K27me3 gains in their promoter. Surprisingly, there was no significant difference in global occupancy of histone modifications across IDHMu and IDHWT. However, comparisons of regulatory landscape identified by H3K4me1 across IDHMu, IDHWT, and normal blood cells revealed an IDHMu unique regulatory regions enriched in PU.1 binding site associated with leukemogenesis.

Conclusions: Our results support a model where IDH neomorphic mutations lead to localized reprogramming of the epigenetic landscape and rearrangement of regulatory regions in primary AML.

[63] Regulatory Regions Distal to *Sfmbt2* Revealed by e4C Analysis May Represent Novel Allele-Specific Enhancers of Transcription in Trophoblasts

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Sfmbt2 is a maternally imprinted gene encoding a polycomb-group protein critical for extraembryonic tissue development and derivation of trophoblast stem cells (TSC) in *Mus musculus*. Lack of paternal *Sfmbt2* results in reduced placenta size and pre-term mortality. Paternal-specific H3K27ac and H3K4me1 peaks along with maternal-specific H3K27me3 peaks at the *Sfmbt2* promoter proximal region reflect the paternal-specific expression patterns of *Sfmbt2* in TSCs. Although *Sfmbt2* is highly expressed in TSCs, cell-type specific regulation of *Sfmbt2* transcription remains poorly understood.

We performed a genome-wide screen for looping events with the *Sfmbt2* promoter (e4C) to identify novel distal regulatory elements controlling *Sfmbt2* expression in TSCs and examined preferential interactions with allele-specific 3C (AS3C). The paternal *Sfmbt2* promoter preferentially interacts with a contact region 60kb upstream that is classified as a putative enhancer due to enrichment in H3K27ac and H3K4me1. A proximal region 250kb downstream of *Sfmbt2* interacts more frequently with the *Sfmbt2* promoter on the maternal allele. The downstream region is characterized by repressive H3K27me3 marks, and lacks H3K27ac and H3K4me1 signals. Cdx2 and CTCF bind the putative enhancer suggesting this enhancer regulates target genes through a Cdx2 dependent mechanism. Distal to *Sfmbt2*, interactions between *Sfmbt2* promoter and euchromatic domains were enriched on the paternal allele whereas the maternal *Sfmbt2* promoter was associated with heterochromatic domains in TSCs. This combination of e4C, AS3C and peak analysis indicates that the paternal *Sfmbt2* promoter is in an open chromatin conformation and loops out to upstream putative enhancer regions whereas the maternal *Sfmbt2* promoter is compacted and prefers interacting with the proximal downstream region.

[64] Human-induced pluripotent stem cells models of Kleefstra syndrome

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Kleefstra Syndrome (KS) is a neurodevelopmental disorder characterized by autistic-like features, intellectual disability, hypotonia, microcephaly, seizures, heart and urogenital defects. KS is caused by a microscopic deletion of the chromosomal region 9q34.3 or by mutations in the euchromatin histone methyltransferase 1 (EHMT1). Mouse and Drosophila models of the disease have been reported. However, despite great progress in the study of this disease and its underlying causes, treatment for children with KS is restricted to symptoms, such as medication for treating seizures and speech therapy. Human-induced Pluripotent Stem Cells (iPSCs) have been shown to be good models for drug discovery and study of the disease molecular mechanisms. We have characterized four iPSCs lines of two KS syndrome patients and four iPSCs lines of patients' sex-match siblings. Quality control experiments were performed to assure the bona fide nature of our iPSCs. Moreover, we genetic engineered iPSC lines of the two healthy siblings to simulate the patients' mutations. The first two exons of the EHMT1 SET-domain were deleted and we assessed to repair an EHMT1 point mutation. Our iPSC lines provide a good model for drug discovery, drug toxicity, and clinical translational research.

[65] Role of folic acid supplementation on embryonic developmental delay and global DNA methylation patterns associated with assisted reproduction technologies.

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Assisted reproductive technologies (ARTs), *now commonly used worldwide,* have been linked with adverse perinatal outcomes and an increased incidence of genomic imprinting disorders, often associated with defective DNA methylation. To establish and maintain proper DNA methylation profiles during early embryo development, the availability of methyl donors such as dietary folate is crucial. The objective of this study is to determine whether folic acid supplementation can prevent embryonic development delay and defects in global DNA methylation associated with ART in a mouse model. CF1 female mice were fed diets with different levels of folic acid supplementation: control (2 mg/kg- CD), low level (8 mg/kg- 4xFASD) and high level (20 mg/kg- 10xFASD) for six weeks prior to ART (superovulation, in vitro fertilization, embryo culture, blastocyst transfer) or natural mating. Diets were continued until collection of mid-gestation embryos and placentas. DNA methylation patterns were examined on a genome-wide basis using Reduced Representation Bisulfite Sequencing (RRBS) on six representative female embryos and placentas per group. A greater proportion of delayed embryos was observed in the ART group fed the control diet (CD) compared to the natural mating group (NAT) fed the same diet (24.5% vs 5.5%, p=0.02). The 4FASD group exhibited a significant decrease in the proportion of delayed embryos compared to the CD group (10.8% vs 24.5%, p=0.03) which was not the case for the 10FASD group (26.1% vs 24.5%, P>0.05). Preliminary analysis of the RRBS demonstrated both increases and decreases in DNA methylation from all comparisons following ART procedures and folic acid supplementation. In particular, results in the embryos showed that ART induced mainly hypomethylation (1225 differentially methylated tiles or DMTs), which was corrected by both doses of folic acid supplementation (229 and 257 DMTs). The same correction was observed in delayed embryos, with more hypomethylated tiles being detected (CD: 6705, 4FASD: 1853 and 10xFASD: 2098 DMTs). The placenta exhibited a higher susceptibility to ART, with 9600 hypomethylated tiles, which decreased to 610 in the 4FASD group while 1518 tiles remained hypomethylated in the 10xFASD group. Taken together, these results suggest that folic acid supplementation at low dose has protective effects on embryonic developmental delay and global DNA methylation defects but that the use of higher dose, comparable to the one used for high risk pregnancies, is not as beneficial. (Supported by CIHR)

[66] RNA Polymerase II stalling targets histone acetylation to transcribed genes.

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Acetylation of histone proteins is a hallmark of transcriptional activity in eukaryotes. In humans, it is associated with multiple diseases characterized by aberrant gene expression, and the enzymes controlling acetylation are key targets for therapeutics. Therefore, characterizing the mechanisms regulating histone acetylation is imperative for understanding both disease development and treatment. A crucial mechanistic question is whether the link between acetylation and transcriptional activity is of a causal or consequential nature, and despite decades of research this is still a matter of debate. Here, through genome-wide analysis in human cells and in *S. cerevisiae*, we reveal that the majority of histone acetylation is dependent on RNA polymerase. Continuing in *S. cerevisiae* we find that while being necessary, histone acetyltransferase (HAT) recruitment to gene bodies is not sufficient for histone acetylation, and uncover that HAT activity is predominantly found in areas of RNAPII stalling. Moreover, consistent with a role of nucleosomes in obstructing progression of RNAPII, previously published algorithms that predict nucleosome stability based on sequence alone can be used to predict histone acetylation. Finally, we show that genetically inducing RNAPII stalling via overexpression of a dominant negative TFIIS mutant promotes histone acetylation. These findings suggest that a role of acetylation in transcription is a response to, and potentially involved in bypass of, nucleosome-induced transcriptional stalling.

[68] Signal-specific function of P-TEFb complexes in the development of cardiac hypertrophy

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Heart disease is one of the leading causes of mortality and requires a further understanding of the underlying etiology to develop better therapeutics. Cardiac remodeling and hypertrophy associated with heart disease develop due to prolonged mechanical stress and hormonal signaling. The remodelling process initially preserves cardiac function, but is ultimately maladaptive and leads to heart failure. The hypertrophic response is characterized by an increase in cardiomyocyte size and gene expression changes; both of which are dependent on the activity of positive transcription elongation factor b (P-TEFb). Cdk9, the kinase subunit of P-TEFb, phosphorylates RNA polymerase II (RNAPII) and other components of the transcriptional machinery leading to the release of RNAPII from a promoter-proximal state to productive elongation resulting in an increase in transcription. Hormonal signals that increase cardiomyocyte size and P-TEFb activity include various agonists for G protein-coupled receptors (GPCRs). Receptor activation initiates signalling cascades that increase formation of two primary active P-TEFb complexes, one containing the bromodomain and extra-terminal (BET) protein Brd4 and another, larger complex termed the Super Elongation complex (SEC). The relative contributions of these complexes to the hypertrophic response, and how they respond to distinct GPCRs, are not known. We hypothesized signaling through distinct GPCRs might elicit similar morphological and gene expression changes, but lead to differential activation of P-TEFb complexes and gene regulatory mechanisms. As expected, RNA-seq analysis showed that genes upregulated by the α_1-adrenergic receptor or endothelin receptor agonists phenylephrine and endothelin-1 respectively, were dependent on Cdk9 activity, but phenylephrine-modulated genes had a greater reduction in expression following Cdk9 inhibition. Disruption of the SEC, through RNAi-mediated knockdown of Aff4, indiscriminately prevented the hypertrophic response to either agonist. However, preventing Brd4-mediated recruitment, through RNAi or a small-molecule inhibitor, only reduced the hypertrophic response to phenylephrine. As confirmed by chromatin immunoprecipitation, recruitment of Brd4 only occurs in response to phenylephrine. We are currently investigating a novel mechanism of Brd4 activation due to signaling unique to the α_1-adrenergic receptor leading to the differential Brd4 recruitment observed. Complementary studies *in vivo* will further define the role of Brd4 in orchestrating the hypertrophic response to distinct stimuli. The activation of the Brd4/P-TEFb complex in response to selective hypertrophic stimuli has potential clinical implications as therapies targeting Cdk9 activity and recruitment are currently being explored for heart failure.

[72] Targeting EZH2 reactivates a breast cancer subtype-specific anti-metastatic transcriptional program

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Emerging evidence has illustrated the importance of epigenomic reprogramming in cancer, with altered post-translational modifications of histones contributing to the pathogenesis of many tumor types including breast cancer. However, the contributions of histone modifiers to breast cancer progression are unclear, and how these processes vary between molecular subtypes has yet to be adequately addressed. In this study, we demonstrate that genetic or pharmacological targeting of the epigenetic modifier Ezh2 dramatically hinders metastatic behavior in a mouse model of breast cancer reflective of the LuminalB subtype. We also employ patient derived xenografts from different intrinsic subtypes of breast cancer to confirm that pharmacological inhibition of Ezh2 activity hinders metastasis specifically in the Luminal B subtype. We further define a molecular mechanism intrinsic to the Luminal B subtype whereby EZH2 maintains H3K27me3-mediated repression of the FOXC1 gene, thereby inactivating a FOXC1-driven, anti-invasive transcriptional program. We demonstrate that higher FOXC1 levels are predictive of favorable outcome specifically in Luminal B breast cancer patients and establish the use of EZH2 methyltransferase inhibitors as a viable strategy to block metastasis in Luminal B breast cancer, where options for targeted therapy are currently limited.

[73] Single-Nucleus transcriptomes and methylomes from the brains of depressed suicide completers

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Purpose: Molecular changes are typically measured in tissue homogenates or cellular fractions containing millions of cells pooled together. However, most tissue types, particularly the brain, have heterogeneous cellular composition. Multiple neuronal and glial subtypes with specific gene expression patterns are likely to be distinctly modified in a diseased state. As such, the normal variation of gene expression between cell types can mask specific changes when obtaining molecular information from cellular homogenates. This principle is particularly true for DNA methylation profiles, where cell type-to-cell type variability is greater than inter-individual variability. The detection of subtle transcriptomic and methylomic alterations such as those expected from psychiatric disease will benefit greatly from single cell resolution.

Methods: Using post-mortem brain tissue obtained from the Douglas Bell Canada Brain bank we explored the frontal cortex of 16 suicide completers and 16 matched healthy controls. Bulk nuclei were enriched from BA8/9 and single nuclei were captured using a droplet-based protocol. The droplet-based capture allowed for the capture of 3000 nuclei per samples. Whole methylome sequencing was also performed on single nuclei isolated by Fluorescence Assisted Cell (FAC) sorting. Each single nucleus transcriptomic profile was grouped using a graph-based clustering approach. Given that gene body mCH and transcript abundance are for the most part, inversely correlated, each individual methylome was assigned to a transcriptome classified group based on the minimum spearman correlation coefficient.

Results: Using this single nuclei approach, we can identify unique transcriptomic subtleties between groups. Furthermore, the combination of single nuclei transcriptomic and methylomic data allows for a unique depth of analysis not previously attainable in complex disease states. Though, the features that cause these differences need further study, these data are the stepping stone for identifying specific genes and networks whose expression is altered in depression.

[74] Delineating the functional diversity of KDM6 family histone demethylases UTX and JMJD3 during muscle regeneration

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Methylation dynamics on histone and non-histone proteins is one of the most critical post-translational modifications during embryonic development and cellular differentiation. Lineage-determination genes involved in the developmental processes are silenced by trimethylation marks at histone H3 lysine 27 (H3K27me3). The KDM6 family proteins UTX (KDM6A) and JMJD3 (KDM6B) remove H3K27me3 marks to permit gene expression. UTX and JMJD3 proteins both harbor a highly homologous Jmjc domain that facilitates H3K27me3 demethylation, conserved c-terminal domains and divergent N-terminal domains. Distinguishing UTX from JMJD3 is the presence of a tetratricopeptide repeats (TPR) motif that is predicted to mediate protein-protein interactions). The presence of the TPR motif in UTX suggests the KDM6 family members may interact with distinct sets of proteins. Our laboratory has previously shown that UTX is recruited to upstream regulatory regions (enhancers and promoters) to remove repressive H3K27me3 demethylation mark on multiple muscle genes. This ability to remove H3K27me3 marks at muscle genes is essential for muscle formation, as mice lacking UTX cannot regenerate their muscle fibers in response to muscle injury. Interestingly, muscle progenitor cells also express JMJD3, suggesting that the two KDM6 demethylases may play divergent roles in muscle regeneration. Using shRNA mediated knockdown, we find that JMJD3 plays an essential role in the differentiation in primary myoblasts that is not redundant with UTX. ChIP experiments revealed an inverse correlation in the occupancy of UTX and JMJD3 on various muscle genes during myogenesis. Our characterization of the differential roles of UTX and JMJD3 in the regulation of muscle regeneration is ongoing, and our latest findings will be presented.

[76] Identification of frequently mutated regulatory regions in human breast cancer

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Human breast cancer is genomically complex with many poorly understood alterations in non-coding regions of the genome. Here we describe a study to address the possibility that specific genomic regulatory regions active in normal cells may be frequently mutated in breast cancer and may modulate gene expression. First, we identified active regulatory regions in the genome of each of the 3 phenotypically separable and biologically distinct subsets of normal human mammary epithelial cells that can be routinely isolated from reduction mammoplasties. Active regulatory regions were defined as those positive for H3K27ac together with H3K4me1 or H3K4me3 as identified in ChIP-seq experiments carried out on these same purified cell types. We then used a recently published dataset of mutations found in 560 breast cancers to measure the degree of enrichment for these mutations across all regions of non-coding DNA. Generally, regulatory regions were depleted of non-coding mutations compared to the rest of the genome. However, a few regions in each cell type were frequently mutated in breast cancers (FDR<0.05). Integration of these results with gene expression data obtained from the same tumours showed some mutations in these regions correlated with significantly altered expression of the associated genes, including *KLF6* and *BATF3*. These results show that subsets of frequently mutated regulatory regions can be associated with altered gene expression in human breast cancers. They also lay the foundation for future characterization of the specific mutations identified, and their potential role in breast cancer development and progression.

[77] Using a transgenic mouse model combined with an environmental challenge (high-fat diet) to delineate molecular mechanisms implicated in epigenetic inheritance

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Within the last four decades, an obesity epidemic has spread world-wide. It is well established that genetic and lifestyle factors are responsible for the development of obesity and other complex diseases. Nonetheless, epidemiological studies and diet-induced obesity rodent models have shown that overweight fathers are more likely to have overweight children, even if the child consumes a healthy diet. The underlying molecular mechanisms are thought to involve developmental origins of adult disease and epigenetic inheritance, but are poorly defined. Spermatogenesis involves tightly regulated changes in chromatin that establish heritable information transmitted via the sperm to the embryo. The epigenome establishment in spermatogenesis may be influenced by paternal diets that impact methyl donor availability leading to altered sperm epigenome and affect offspring health. We aimed to investigate the role of histone methylation in sperm in response to diet, and offspring phenotypes. The Kimmins lab previously established a transgenic mouse model that overexpresses KDM1A, an enzyme responsible for demethylation of histone H3 lysine 4 mono- and di-methylation (H3K4me1/2) (Siklenka et al., 2015; *Science*). Expression of the transgene in the germline gives rise to sperm with an altered epigenome, which negatively impacts offspring development and survivability. In this model, H3K4me3 is also altered in sperm at genomic regions involved in metabolism and development, and intriguingly a subset of sites escapes epigenome reprogramming between generations.

We hypothesized that by challenging the KDM1A transgenic mice with a high-fat diet, we will identify environmentally sensitive regions that escape reprogramming in sperm. We aimed to determine whether H3K4me3 is implicated in epigenetic inheritance in the transmission of obesity-induced metabolic alterations across generations, and determine whether there will be cumulative deficits in metabolic dysfunction in the offspring, resulting from sires high-fat diet and challenged sperm epigenome.

Transgenic, wildtype littermates and C57BL/6NCrl control males (n=10-13 per group) were fed either a low- or high-fat diet (10% or 60% kcal fat respectively) for 12 weeks starting at weaning. Males were then bred to 8-week-old chow-diet fed C57BL/6NCrl females. All offspring were fed a chow diet. Metabolic function was assessed by baseline and intraperitoneal glucose and insulin tolerance tests for males on the diets and their offspring. High-fat fed males became obese with glucose intolerance, reduced insulin sensitivity, and elevated fasting blood glucose. Sex- and genotype-specific metabolic disturbances in offspring associated with paternal diet were observed. The paternal sperm epigenome was quantitatively assessed by ChIP-sequencing targeting H3K4me3 and epigenetic changes associated with diet were identified. These findings implicate histone methylation in sperm in environmentally induced heritable phenotypes.

[79] Hippocampal progenitor cell models in deciphering the epigenomics of stress.

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Objectives: Exposure to early life stress (ELS) is a well-known major risk factor for developing psychiatric and behavioural disorders later in life. A growing body of evidence indicates that exposure to ELS can lead to long lasting changes in a number of systems including the endocrine system, the immune system and brain structure and function. However, our understanding of the mechanisms underlying these effects is limited. One proposed mechanism that might lead to some of these long-lasting effects is that excessive glucocorticoids (GC) release after ELS exposure induces long-lasting epigenetic alterations in important regulatory genes. Indeed, accumulating evidences suggest that epigenetic mechanisms are in part responsible for the embedding of ELS where the type and timing of stress exposure are important moderating factors.

Methods: We used human hippocampal progenitor cells (HPCs) exposed to GCs during neurogenesis and multi-omic data analysis integrating gene expression and DNA methylation (5mC) at a genome-wide level to assess the long-lasting effects of GCs.

Results: We identified long-lasting 5mC alterations induced by GCs exposure during neurogenesis, where a significant portion of these marks were maintained after neuronal differentiation. Moreover, the sites showing GC-induced methylation changes are enriched in regulatory regions as well as in genes differentially methylated during fetal brain development and genes previously associated with child abuse in human hippocampus and blood cells. To some extent, they also reflect epigenetic changes induced by acute GCs exposure in human blood cells.

Conclusion: Together, these results suggest that GC-induced epigenetic alterations in HPCs might reflect GC actions during ELS and be in part responsible for the increased risk for psychopathology.

[80] Tissue-specific Epigenetic Changes Associated with an Altered Adult Glucose Metabolism in a Mouse Model of Intrauterine Growth Restriction (IUGR).

Bethany Radford and Victor Han

IUGR is a pregnancy condition where the fetus fails to grow to his/her full potential, resulting in an increased perinatal and neonatal morbidity and mortality; and as adults an increased risk for type II diabetes. Our laboratory has established a maternal nutrient-restriction mouse model of IUGR, in which 20% of the 6-month male offspring develops glucose intolerance. Insulin secretion at the time of glucose tolerance testing did not differ, suggesting peripheral insulin resistance. We hypothesize that the altered glucose metabolism is caused by epigenetic modifications established *in utero* in liver, adipose tissue or skeletal muscle in response to fetal nutrient restriction, resulting in gene expression changes that persists into adulthood. Mated female CD-1 mice were randomly assigned to control or nutrient-restricted (MNR) groups. MNR females received 70% of calories consumed by an average *ad libitum* fed dam from E6.5 to 18.5. All offspring were cross-fostered to *ad libitum* fed moms and weaned males were fed *ad libitum* standard chow. To identify which tissues epigenetic changes occurred in, a pyruvate challenge and hepatic portal vein insulin challenge was performed; and serum peptide markers for obesity and diabetes were assayed. Genes candidates for epigenetic modifications were screened using RNA*seq* in six-month tissues. Liver and adipose tissue pAkt-to-Akt ratio in response to insulin was 3 and 3.6 folds lower, respectively, in MNR offspring (*p*-value = 0.008) indicating insulin resistance in these tissues. MNR offspring also had higher serum PAI-1 at one (*p*-value = 0.04) and six months (*p*-value = 0.04), and resistin at 6 months (*p*-value = 0.04). Although metabolic assessments suggested fetal undernutrition impacted liver and adipose tissue of MNR offspring, gene expression data at six months did not indicate candidate genes that would be underlying these metabolic disturbances. These results suggest that metabolic changes in male nutrient-restricted offspring are not mediated by persistent gene expression changes. Further analysis of the metabolome may indicate gene candidates for epigenetic changes *in utero*.

[81] Effect of Folic Acid Supplementation on Adverse Morphological Outcomes and Imprinted Gene Methylation in Offspring Conceived Using Assisted Reproduction

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Several adverse outcomes have been associated with the use of assisted reproductive technologies (ART), techniques responsible for the conception of up to 6% of children around the world. DNA methylation profiles established during germ cell and early embryo development have been shown to be vulnerable to these procedures. Importantly, folate from the diet contributes methyl groups essential for the proper establishment of these epigenetic patterns. The goal of my research is to elucidate whether folic acid supplementation can prevent the negative outcomes associated with ART, including birth defects and epigenetic aberrations. Outbred female mice were fed control (2 mg folic acid/kg; CD), low dose (8 mg folic acid/kg; 4FASD) or high dose (20 mg folic acid/kg; 10FASD) folic acid-supplemented diets six weeks prior to assisted reproduction and diets were continued throughout gestation. All diet groups displayed similar levels of embryo viability; however, we observed higher levels of overall and specific embryonic malformations in the 10FASD group. Next, we examined imprinted gene methylation in midgestation embryos and placentas by bisulfite pyrosequencing. ART resulted in imprinting defects in the placenta at both the *Snrpn* and *H19* differentially methylated regions (DMRs), which was not rescued by the folic acid-supplemented diets. In the embryo, the methylation level at the *Snrpn* DMR was not affected by ART and was similar between diet groups. Conversely, at the *H19* DMR, not only were methylation levels in embryos lower in the CD group compared to naturally-mated mice fed CD (NAT-CD), but the 10FASD group demonstrated significantly lower methylation than the 4FASD group, indicating a dose-dependent effect. Notably, for both DMRs, ART resulted in a significant increase in variance in both the embryo and the placenta. In the placenta, the 10FASD was associated with an increase in variance at the *Snrpn* DMR, whereas the 4FASD led to decreased variance at the *H19* DMR. Similarly, in the embryo, the 4FASD group demonstrated lower variance at the *Snrpn* and *H19* DMRs, and the 10FASD group exhibited greater variance at the *H19* DMR exclusively. Together the embryo morphology and epigenetic studies suggest that low dose folic acid may be beneficial while high doses are deleterious in ART pregnancies in a mouse model. This study is helping to elucidate the effects of clinically-relevant folic acid supplementation on pregnancies conceived by ART. (Supported by CIHR and Centre for Research in Reproduction and Development (CRRD))

[82] The subunits *trithorax* and *Menin 1* of the epigenetic regulating TRX complex are necessary for long term memory in *Drosophila melanogaster*

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Epigenetic regulation is important for the initiation and maintenance of the gene expression changes required for long term memory. One specific epigenetic mark required for memory is the trimethylation of Histone H3 Lysine 4. In *Drosophila melanogaster,* the gene *trithorax* (*trx*) is capable of producing this mark but its role in memory has yet to be identified. Here I used RNAi to knockdown *trx* specific to the fly memory control region, the mushroom body (MB). I found that MB-specific *trx* knockdown flies have long term memory deficits, but display normal short-term memory. I have also found that a partner of trithorax in the TRITHORAX complex (TRX complex), Menin 1 (Mnn1), shares a similar phenotypic pattern in memory when knocked down. Currently, using a temporally controlled RNAi knockdown system, I am conducting experiments to discern whether *trx* and *Mnn1* knockdowns affect memory through developmental mechanisms, or through acute effects on gene regulation in the adult brain. Subsequently, I will use MB-specific RNA-sequencing to identify genes and pathways that are misregulated in *trx* knockdown MBs. This study has identified *trx* and *Mnn1* as specific regulators of LTM in *Drosophila*, and will help to further understand the specific epigenetic mechanisms that are important in learning and memory.

[83] Recognition of human elements regulating escape from X-chromosome inactivation in mouse

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X-chromosome inactivation (XCI) epigenetically silences one X chromosome in every cell in female mammals; however, approximately 12–20% of genes escape from XCI in humans and continue to be expressed from both the active and inactive X chromosomes, while only 3–7% of X-linked genes escape in mouse. In light of known species differences between escape gene number and distribution on the X chromosome, we tested whether mouse is a suitable model for studying human escape from XCI through integration of a human BAC at the mouse X-linked *Hprt* gene. Subsequent expression and DNA methylation analysis of transgene activity on an inactive X chromosome in adult female mice demonstrated expression and correspondingly low promoter DNA methylation of human genes *RPS4X* and *CITED1*. Therefore, either the mechanisms of escape are conserved between mouse and human, or at the very least the mouse system is capable of recognizing human elements necessary for escape from XCI. Silencing of a normally subject gene (*ERCC6L)* on the BAC, as well ongoing silencing of genes near the integration site, also suggests retention and recognition of boundary elements between subject and escape regions on the BAC. Preliminary data from developmental time points suggests that transgenic *RPS4X* is expressed from the inactive X in females as early as embryonic day 9.5, arguing for continuous expression from the transgene rather than initial silencing and reactivation.

As human ESCs remain an epigenetically unstable model for studying XCI, we have generated a female mouse ESC line with an *Hprt* “docking site” similar to the transgenic mouse system, allowing more rapid assessment of additional human genes for intrinsic escape ability; as well as facilitating further investigation and characterization of the critical regions responsible for continued expression from an inactive X chromosome. Expression of genes escaping from XCI may contribute to the phenotypes of X aneuploidies as well as influence male and female susceptibility to disease. In an attempt to harness the potential of the second allele to protect from X-linked disease in females, recent studies have explored targeted reactivation of the inactive X but have tended to reactivate a substantial portion of silenced genes, therefore, further understanding of how gene regulatory mechanisms work the X might provide insights into utilization of such therapeutic approaches.

[85] A synthetic acetylation substrate to study Gcn5 targeting and function in yeast.

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The SAGA (Spt-Ada-Gcn5 Acetyltransferase) complex is a highly conserved transcriptional coactivator complex responsible for the regulation of numerous cellular processes, including histone acetylation. The SAGA complex is comprised of ~20 subunits organized into 4 submodules. Gcn5 is the catalytic enzyme of the histone acetyltransferase (HAT) module of the complex. Gene transcription resulting from histone acetylation has been shown to be dependent on the presence of many of these subunits, and lack thereof results in a downregulation of distinct subsets of genes. Recent advancements in protein analysis technologies have shown that acetylation also occurs on non-histone targets, to the extent that nearly one third of all proteins can be acetylated in the budding yeast *Saccharomyces cerevisiae*. Despite this, very few non-histone targets of acetylation have been fully characterized, and even less is known about how HAT enzymes choose their substrates.

Previous work from our group has proposed a Gcn5 specific consensus sequence, based on high confidence regulated sites following large scale proteomics. I hypothesize that recognition of a specific consensus sequence is required for Gcn5-dependent acetylation, and addition of one will transform a known non-substrate into a target. I generated a synthetic substrate where the Gcn5 specific consensus sequence SSKRP was fused onto the C-terminal end of GFP, a known non-substrate of Gcn5. Using IP/Western directed techniques, I showed that addition of the consensus sequence is sufficient to confer acetylation of a non-substrate by Gcn5.

My goal is now to exploit our synthetic substrate and the Gcn5 consensus sequence as a tool to probe Gcn5 and SAGA biology *in vivo*. First, I determined which SAGA subunits of the SAGA complex are required for acetylation of this synthetic non-histone substrate. My results indicated that Ada3, a member of the HAT submodule, is a key regulator of our synthetic substrate. Large scale SILAC based proteomics confirmed these results are not unique to our substrate and identified over 100 novel acetylation sites regulated specifically by Ada3. Second, I searched for the Gcn5 consensus sequence in native proteins to identify new substrates. Using this approach, we have characterized the Spt2 transcriptional regulator as a novel target of Gcn5. I conclude that target sequences are important for non-histone substrate targeting by the Gcn5 HAT and that synthetic substrates provide a novel way to study HAT complexes *in vivo*.

[86] Recovery of prepubertal thyroxine levels in Atrx Foxg1Cre mice does not rescue Igf1 expression or improve aging-like phenotypes

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α-Thalassemia/mental retardation X linked (ATRX) is a chromatin remodeling protein of the Swi/Snf family involved in deposition of the histone variant H3.3 at repetitive sequences including telomeres. ATRX regulates several genes and is important for maintaining genomic stability which is an important determinant of lifespan. ATRX deletion in the embryonic forebrain and anterior pituitary (FoxG1 Cre) results in decreased life span, subcutaneous fat, serum glucose levels and myelination with bone and growth abnormalities. These mice also exhibit hormone deficits characterized by low thyroxine (T4) and insulin-like growth factor 1 (IGF1) These phenotypes are characteristic of premature aging. **I hypothesize that, supplementation of *Atrx* cKO mice with T4 will result in an increase in IGF1 and rescue premature aging phenotypes.**

Supplementation of T4 does not rescue reduced growth, lifespan, subcutaneous fat or serum glucose caused by ATRX deficiency. Circulating levels of T4 are increased in mutant mice supplemented with T4, however, IGF-1 levels are not. Furthermore, expression of some thyroid hormone-responsive genes, including Igf1, are not restored to control levels after T4 treatment. When control mice were injected with T4, these genes exceeded control levels indicating *Atrx* may be involved in their expression. Unexpectedly, there was a significant decrease in the number of cells expressing ATRX in cKO mouse livers. This is due to the activation of Cre recombinase and subsequent deletion of *Atrx* in the liver. This data suggests that ATRX is required for the expression of some thyroid hormone responsive genes in the liver. Deletion of *Atrx* from hepatocytes in the liver (Albumin Cre) results in a decrease in Igf1 as well as all other genes suspected to be regulated by *Atrx*. However, AlbCre cKO mice do not exhibit premature aging phenotypes despite low Igf1 transcripts and in fact have normal levels of circulating IGF1.

I conclude that ATRX is involved in the regulation of a subset of thyroid hormone responsive genes, including Igf1. T4 injections in the early postnatal period did not rescue premature aging phenotypes due to a failure to restore IGF1 to control levels. Deletion of *Atrx* in hepatocytes results in a decrease in all genes suspected to be regulated by ATRX providing further evidence that *Atrx* is important for the regulation of a subset of thyroid hormone responsive genes in the liver.

[87] Exosomal microRNAs as a biomarker for antidepressant response

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Background: Major depressive disorder (MDD) is a common psychiatric disorder affecting millions of people worldwide. The treatment for MDD is up to the discretion of the physician and is, therefore, highly subjective. Furthermore, patients often do not respond to the first antidepressant they are prescribed. As a result, biomarker discovery has become an important and prominent aspect of MDD. However, the majority of research in this field is limited to peripheral tissue, leaving questions concerning the relavence of these findings in the context of pyschiatry disease. A potential solution to this hurdle is exosomes — small extracellular vesicles that contain the cytosolic contents of their cell of origin. The exchange of its contents, largely small RNA molecules, can exert an influence on its target cell. Neural- derived exosomes (NDE) and their contents can provide valuable regulatory information from the brain in peripheral tissues.

Methods: This pilot study used 2 mls of plasma from 10 control samples and 10 patients with MDD, including 5 patients who did not respond to antidepressant treatment in a longitudinal follow-up. For each sample, exosomes were isolated using a size exclusion column from Izon Science (Christchurch, New Zealand). Each plasma isolation was divided in half to produce a “whole exosomes” fraction and, an immunoprecipitated “(NDE)” fraction using the neural marker L1CAM. An aliquot of each fraction underwent quantitation and size determination using Tunable Resistive Pulse Sensing (TRPS) on the gNano gold. The remainder of the sample was used to extract small RNA and sequenced using the Illumina platform.

Results: We have obtained exosome concentrations that are consistent with what is expected from the literature, and we have successfully constructed small RNA library from both whole exosome and NDE fractions. Preliminary data suggests that exosomes from depressed patients are significantly smaller than controls. In addition, neuronal derived exosomes from patients responding to treatment are significantly smaller than both non-responders and controls. We have also produced a preliminary miRNA profile from plasma exosomes in MDD.

Conclusions: Isolating NDE from plasma provides a very valuable resource for biomarker discovery in MDD. Our ongoing work aims to provide a neural miRNA profile in MDD, including profiles of drug response and non-response.

[88] The bromodomain-containing protein IBD1 links multiple chromatin related protein complexes to highly expressed genes in *Tetrahymena thermophila*.

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The chromatin remodelers of the SWI/SNF family are critical transcriptional regulators. Using affinity purification coupled to mass spectrometry (AP-MS) we identified members of a SWI/SNF complex (SWI/SNF^{Tt}) in *Tetrahymena thermophila*. SWI/SNF^{Tt} is composed of 11 proteins, Snf5^{Tt}, Swi1^{Tt}, Swi3^{Tt}, Snf12^{Tt}, Brg1^{Tt}, two proteins with chromatin interacting domains and four proteins without orthologs to SWI/SNF proteins in yeast or mammals. SWI/SNF^{Tt} localizes exclusively to the transcriptionally active macronucleus (MAC) during growth and development, consistent with a role in transcription. Recognition of lysine acetylation through a bromodomain (BRD) component is key to SWI/SNF function; in other species, this function is attributed to SNF2/Brg1. While *Tetrahymena* Brg1 does not contain a BRD, our AP-MS results identified a new BRD-containing SWI/SNF^{Tt} component, Ibd1, that associates with SWI/SNF^{Tt} during growth but not development. AP-MS analysis of epitope-tagged Ibd1 also revealed it to be a subunit of several additional protein complexes, including the putative SWR^{Tt}, and SAGA^{Tt} complexes as well as a putative H3K4-specific histone methyl transferase complex. Recombinant Ibd1 recognizes acetyllysine marks on histones correlated with active transcription. ChIP-Seq analysis suggests Ibd1 functions in transcription. We suggest Ibd1 coordinates the recruitment of several chromatin remodeling complexes to promote transcription of highly expressed genes in *Tetrahymena*.

[89] Investigating epigenetic regulation of the adenovirus genome as a potential therapeutic target in infection.

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The human adenovirus (Ad) causes minor respiratory illnesses in most patients, but can lead to severe disease and death in pediatric, geriatric and immunocompromised patients. No approved antiviral therapy currently exists for the treatment of severe Ad-induced diseases.

Within the first few hours of infection, the Ad DNA enters the host cell nuclei and associates with cellular proteins including histones, adopting a nucleoprotein structure similar the cellular DNA. Assembly of the viral genome into this repeating nucleosome-like structure is crucial for efficient expression of virus-encoded genes, and it allows viral gene expression to be regulated by the same epigenetic mechanisms as the host. Consequently, one approach to treating Ad-induced disease may be to prevent the viral DNA from transitioning to this “chromatinized”, transcriptionally active state. Thus, our objective is to investigate the epigenetic regulation of Ad gene expression, and identify cellular epigenetic modifiers that can serve as novel therapeutic targets to abrogate Ad infections.

Using a wildtype-like, red fluorescent protein-expressing Ad construct, we have designed and validated an efficient method to screen small molecules that modulate the activities of cellular epigenetic modifiers. Through a preliminary screen, we identified the pan-histone deacetylase (HDAC) inhibitor vorinostat as a compound that effectively reduced Ad replication. Follow-up studies revealed that vorinostat significantly delays the onset of viral gene expression and replication, and that this is mediated through the inhibition of HDAC2 activity. Further elucidation of the underlying mechanism and in vivo studies are underway. We have also screened the Cayman Epigenetics Screening Library and identified several compounds as potential inhibitors of Ad. We are currently validating these positive hits.

The costs associated with Ad-induced disease are significant in terms of medical expenses, lost work hours and loss of life in some populations. Identifying the role of epigenetic modifiers in Ad infections will not only reveal novel pathways for therapeutic intervention, but also provide novel insights into virus-cell interactions in Ad infections, which may be relevant to other DNA virus infections.

[90] Expansion of BioID toolbox for study of protein interactome in primary cells

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Proximity-dependent biotinylation strategies have emerged as powerful tools to characterize the subcellular context of proteins in living cells. The popular BioID approach employs an abortive *E. coli* biotin ligase mutant (R118G; denoted as BirA*), which when fused to a bait protein enables the covalent biotinylation of endogenous proximal polypeptides. This approach has been mainly applied to the study of protein proximity in immortalized mammalian cell lines. To expand the application space of BioID, here we describe a set of lentiviral vectors that enable the inducible expression of BirA*-tagged bait fusion proteins for the implementation of BioID in diverse experimental systems, including terminally differentiated primary cells as well as pluripotent embryonic stem cells. Using these reagents that we have benchmarked, we are exploring the interaction network of a number of chromatin-associated factors, such as transfection factors, epigenetic regulators and histone variants. Through iterative profiling of the candidate baits proteins using lentiviral mediated BioID in various cell types, we aim to identify the constitutive and cell type specific interactomes epigenetic regulation, providing insight into transcriptional regulatory mechanisms.

[91] Elucidating the role of DNA methylation in continuous phenotypic variation

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Background: Within promoters, the presence of methyl groups on the fifth carbon of cytosines is strongly associated with transcriptional repression. The genome-wide pattern of DNA methylation is tightly regulated and is established in a cell-type specific manner. Yet, it is the dynamic and reversible nature of DNA methylation that allows environmental inputs to produce transcriptional changes that can be maintained through cell division. As genetic variation has failed to explain all of the variance in continuous traits, it is hypothesized that this property of DNA methylation allows it to complement genetic variation in producing a continuous spectrum in quantitative traits. However, the relative contribution of each mechanism – especially from a causational perspective – remains unclear. The Florida carpenter ant is a uniquely attractive model organism to dissect these complex interactions due to a high relatedness between individuals that maximizes the need for epigenetic mechanisms to generate size variation, a natural and continuous size variation that is associated with distinct social roles, and a small genome size that is amenable to high-throughput sequencing.
Objectives: In order to develop an understanding of the interplay between the sequence and methylation of DNA, we aim to analyze genome-wide DNA methylation patterns in combination with a genome-wide analysis of DNA sequence variation. By investigating correlations between methylation of particular cytosines and larval size, we will determine the important molecular pathways that are affected by the environment to modulate growth and ultimately regulate division of labor. We will test the hypothesis that changes in nucleotide sequence are not sufficient to explain the observed phenotypic variation. We will also analyze genome-wide mRNA and non-coding RNA transcript levels in order to be able to associate changes in sequence and methylation with functional consequences.
Methods: DNA and RNA will be isolated from 25 ants such that each individual will be subjected to whole-genome sequencing, whole-genome-bisulfite sequencing, and RNA-sequencing. We will develop a novel bioinformatics pipeline to produce high-quality data from each individual ant. We will then use a Pearson correlation to analyze the association of ant size with cytosines that are covered by at least 10 reads, and apply standard analyses for sequence variation and transcript levels. We will also observe how environmental perturbations modify the DNA methylome.
Preliminary Results: A pilot study of 5 ants revealed 547 cytosines with a Pearson correlation coefficient greater than 0.95. The genes nearest to these cytosines were enriched in GO terms that included skeletal system morphogenesis, epithelial cell migration, regulation of histone modifications, and metabolism of fatty acids. The cohort did exhibit appreciable inter-individual genetic variation.
Conclusions: By finding preliminary associations in genes that belong to important pathways in growth, we have demonstrated that our experimental approach – which requires low input DNA from single ants, continuous variation in methylation, and a working bioinformatics pipeline – is capable of producing meaningful methylation data. We will perform the same analyses in a larger population to discover changes that reach genome-wide significance.

[92] Characterizing the ATRX Interactome and its Effect on Chromatin

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The ATRX protein behaves as a multifunctional chromatin remodelling protein that oversees silencing of repetitive DNA elements. The C-terminus of ATRX contains a helicase and ATP-dependent chromatin remodelling domain, while the N-terminus enforces heterochromatin binding through interactions with heterochromatin protein 1 (HP1) and the heterochromatic histone H3.3 modification H3K9me3. ATRX is reported to interact with a number of proteins, including the histone chaperone DAXX with whom it facilitates the deposition of histone variant H3.3 at telomeres and pericentric heterochromatin. ATRX and/or DAXX is often reported truncated or absent in cells using the cancer pathway known as alternative lengthening of telomeres (ALT). Exogenous expression of ATRX in ALT-positive, ATRX-null cancer cells was found to suppress ALT therefore implying an important role for the protein. Despite the broad involvement of ATRX in various biological processes, the exact molecular effects of ATRX on chromatin remain poorly understood.

We wish to better understand ATRX through proximity-based protein labeling. I here make use of proximity dependent biotin identification (BioID) to describe ATRX protein-protein interactions and to identify changes that occur over chromatin when ATRX is either deleted or re-introduced. To this end, ATRX has been fused to the promiscuous biotin ligase, BirA*, allowing for biotinylation, isolation, and identification of interacting and neighbouring proteins. The latter includes known interactors, such as DAXX, proteins involved in DNA damage response, and some novel interactions. Next, we aim to further determine how ATRX and its interacting proteins help shape the telomeric chromatin landscape. Our strategy uses a dCas9-BirA* fusion localized to telomeres thus allowing tagging of telomeric chromatin proteins. Through these studies, we hope to determine key protein-protein interactions that will help us better understand how ATRX remodels and enforces silencing over telomeric chromatin.

[93] EGF induced nucleosomal response pathway and transcriptional programming in cancer

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Introduction: The RAS-mitogen-activated protein kinase (MAPK) pathway has been deregulated in many cancers. Induction of the RAS-MAPK pathway triggers the initiation of transcriptional responses, which drive the cells towards cell proliferation. Mitogen- and stress-activated kinases 1/2 (MSK1/2), act downstream of the MAPK signalling pathway and phosphorylate histone H3 at S10 (H3S10ph) or S28 (H3S28ph) (nucleosomal response) at regulatory region of immediate early genes (IEGs), but the extent of effects and mechanisms has not been completely understood. Our previous studies reported that MSK1/2 inhibition or knockdown in epidermal growth factor (EGF) induced cells prevented cell growth on soft agar, an in vitro measure of metastatic potential. This observation suggested an important role of MSK in tumorigenesis, providing evidence that MSK is therapeutic target in cancer treatment.

Objectives: To investigate the MSK mediated nucleosomal response pathway and IEG expression

Material and Methods: RNA-sequencing, RT-qPCR, ChIP-Assay

Results: To investigate IEGs regulated by the MSK, we have completed transcriptome analyses (RNA-Seq) of human normal (CCD1070SK70), mouse fibroblast (10T½) and colorectal cancer (HCT-116) cell lines stimulated with EGF ± H89, a potent MSK inhibitor. RNA-Seq results validated by RT-qPCR on these cell lines show that MSK mediated nucleosomal response pathway triggers IEG transcription. MSK inhibitor H89 reduced the induction of IEGs. We found many common IEGs altered by MSK mediated signalling. Induction of DUSP1, NR4A1, and EGR3 was prevented with MSK inhibitor H89 in both cancer and normal cell lines. In HCT116 induction of FOS, FOSL1, JUNB and EGR1 was prevented with MSK inhibitor H89 but not in normal cells (CCD1070SK). ChIP assays of human and mouse cells show the transient increase of H3S10ph or H3S28ph at the regulatory regions of IEGs. Our preliminary data show that MSK mediated epigenetic dysfunction alters the regulation of immediate early gene expression in colorectal cancer cells and normal cells. The recent results of our studies on the MSK-mediated nucleosomal response and gene programming will be presented. This research was support by a grant from the CancerCare Manitoba Foundation.

[94] Global landscape and regulatory principles of DNA methylation reprogramming for germ cell specification by mouse pluripotent stem cells

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Specification of primordial germ cells (PGCs) activates epigenetic reprogramming for totipotency, the elucidation of which remains a fundamental challenge. Here, we uncover regulatory principles for DNA methylation reprogramming during in vitro PGC specification, in which mouse embryonic stem cells (ESCs) are induced into epiblast-like cells (EpiLCs) and then PGC-like cells (PGCLCs). While ESCs reorganize their methylome to form EpiLCs, PGCLCs essentially dilute the EpiLC methylome at constant, yet different, rates between unique sequence regions and repeats. ESCs form hypomethylated domains around pluripotency regulators for their activation, whereas PGCLCs create demethylation-sensitive domains around developmental regulators by accumulating abundant H3K27me3 for their repression. Loss of PRDM14 globally upregulates methylation and diminishes the hypomethylated domains, but it preserves demethylation-sensitive domains. Notably, female ESCs form hypomethylated lamina-associated domains, while female PGCLCs effectively reverse such states into a more normal configuration. Our findings illuminate the unique orchestration of DNA methylation and histone modification reprogramming during PGC specification.

[95] Impacts of mucosal environmental factors in regulating immune activation at the female reproductive tract via epigenetic mechanisms

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Background: Epithelium which is a physical barrier, also recognized as a key mediator of host-bacteria symbiosis. Factors involved in this symbiotic relationship include secreted microbial components and cytokines in vaginal fluids (VF). Some may cast long-lasting effects on inducing tolerance to residential microbes. While much is focused on the gut and respiratory mucosae, the female genital mucosa (FGM) where most sexually transmitted infections occur remains understudied. This study explores microbial factors that alter immunity via modifying the epigenetic programing of immunologic genes in epithelial cells from FGM.

Hypothesis: Microbial factors, such as H_2O_2, lactic acid (LA) and Na butyrate (NaB) have direct impacts on the epigenetic regulation of immunologic genes, resulting in altered immune mediators in the VF.

Objectives: We will determine the effects of microbial metabolites, including H_2O_2, LA and NaB on the expression of immunologic genes in vaginal epithelial cells and the secreted cytokine/chemokine levels. The effect of these metabolites will also examine on the epigenetic modification associated with the promoter of immunologic genes and the acetylation of cellular proteins.

Methods: Vaginal epithelial cell lines (VK2) were pre-treated with bacterial culture supernatants, H_2O_2, LA or NaB at optimal, non-toxic concentrations, followed by stimulation with Toll-like-receptor (TLR) agonists. Cytokines RNA and protein were quantitated with RT-qPCR and multiplexing protein assays. Nuclear protein lysates were assayed for HDAC activities and whole cell protein lysates were analyzed for post-translational modifications (i.e., acetylation, methylation and phosphorylation) using proteomic profiling.

Results: Only 2 bacteria (*G. vaginalis* and *L. iners*) among 5 bacterial culture supernatants tested, showed significant up-regulation of IL-1β, IL-8 and TNFα RNA in unstimulated VK2 cells, and only 2 bacteria (*A. vaginae* and *P. bivia*) could modulate the immune response to TLR-3 stimulation in enhancing the secretion of IL-10, MIP-1α, and TNFα. Pre-treating VK2 cells with NaB (5mM) significantly boosted the cellular TNFα RNA in response to stimulations of all TLR(1-9) agonists tested. However, IL-6 RNA expression was boosted by NaB pre-treatment, in response to TLR-3 and -9 stimulation. VK2 cells when exposed to NaB (5mM, 16h) had increased acetylation of 49 proteins, compared to the effects of trichostatin A (TSA, 4nM), which increased acetylation of 100 proteins.

Summary: These findings showed that secreted microbial products, including NaB could modulate the immunologic regulatory function of VK2 cells, and soluble factors from bacteria could affect gene activation in VK2 cells and that NaB and TSA alone can cause changes in protein acetylation (which may lead to changes in protein function). Upon completion, this study will yield insight on the role of epithelial cells in mediating the crosstalk between host and the environment at the FGM.

[96] Autism spectrum disorder associated with mutations in SRCAP upstream of those associated with Floating-Harbor syndrome generate overlapping yet distinct functional DNA methylation signatures

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Autism spectrum disorder (ASD) is an etiologically and phenotypically heterogeneous neurodevelopmental disorder, characterized by impaired social communication, the presence of repetitive behaviours and restricted interests. Genetic causes have been identified (>200 ASD-risk genes), but no single gene mutation accounts for >1% of all ASD cases. A role for epigenetic mechanisms in ASD etiology is supported by the many ASD-risk genes that function as epigenetic regulators and evidence that epigenetic dysregulation can interrupt normal brain development. We have identified two individuals with ASD who carry variants of unknown significance (VUS) in the Snf2 Related CREBBP Activator Protein gene (*SRCAP*). *SRCAP* encodes the core catalytic component of the SRCAP complex, which is involved in chromatin remodelling and acts in the Notch signalling pathway. Heterozygous mutations in exons 33 and 34 of *SRCAP* are known to cause a rare genetic disorder, Floating-Harbor Syndrome (FHS; OMIM #136140), although not all FHS cases have an identifiable genetic cause. FHS is characterized by distinctive facial features, short stature, intellectual and speech impairments, and delayed bone age. ASD is not commonly comorbid with FHS, but the two disorders share some overlapping physical, cognitive and behavioural features. Given that SRCAP functions as an epigenetic regulator within important developmental pathways, we hypothesize that aberrant epigenetic marks contribute to the molecular pathophysiology of FHS and that specific patterns will distinguish between the two phenotypes observed in individuals with independent mutations in the same gene. To test this, we assessed for genome-wide DNA methylation (DNAm) patterns using the Illumina MethylationEPIC array in whole blood DNA from patients with a clinical diagnosis of ASD and *SRCAP* VUS (n=2) or FHS and pathogenic *SRCAP* mutations (n=3). Despite a small number of cases, we identified an FHS-specific DNAm signature comprised of >2,000 CpG sites (FDR corrected p-value<0.001) that distinctly separated FHS cases from age- and sex-matched neurotypical controls (n=59). Pathway analysis performed on the differentially methylated genes in this signature revealed enrichment in neuronal differentiation and fibronectin production, biological pathways relevant to the pathophysiology of FHS. The FHS DNAm signature was used to classify the two individuals with ASD in order to better interpret the pathogenicity of the *SRCAP* VUS, classifying them as intermediate between FHS signature cases and controls, reflecting the partially overlapping phenotypic features described. In summary, we have demonstrated functional and biological utility of the FHS DNAm signature: it can be used as a molecular biomarker to inform pathogenicity of VUS and thus FHS diagnosis, and to elucidate the underlying biological mechanisms that will help us to better understand, diagnose and treat FHS and other disorders with overlapping features, such as ASD.

[97] Soluble mucosal factors can reduce cellular HDAC activities in cervical-vaginal cells to affect an immunologic milieu that is less conducive for leukocyte recruitment and cellular activation.

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Cervicovaginal mucosa consists soluble factors, such as H_2O_2, lactic acid, nitric oxide (NO) and short-chain fatty acids (SCFA). These, together with immune mediators are hypothesized to shape the mucosal immunologic milieu via yet-to-be defined molecular mechanisms. This study examined the role of epigenetic mechanisms in mediating the interaction between the mucosal environment and human cervicovaginal cells.

We found that exposing *primary human* cervical mononuclear cells (CMC) and epithelial cells to low levels of H_2O_2 (50μM) or SCFA (trichostatin A, 2nM or Na butyrate, 100μM) resulted in altered acetylation and phosphorylation of cellular proteins (i.e., histones, heat-shock proteins and proteins in cell-cell binding) and reduced expression of interferon (IFN) effector genes (IFN-γ, CXCL-10, MX-1, IRF-1, IL-12p35) and genes of inflammatory mediator. Curiously, although the SCFA increased histone acetylation at IRF-1 promoter, HDAC2 binding to the promoter was not affected, but IRF-1 transcription was reduced. To investigate the physiologic relevance of these observations, the study thence quantitated the H_2O_2, NO and immune mediator levels in the cervicovaginal lavage (CVL), and the activities of histone deacetylase (HDAC) and acetyl-transferase (HAT), and the RNA level of immunologic genes in the matching CMC from healthy female donors (18-35 years old). Agreeing with *in vitro* data, H_2O_2 level in CVL was inversely correlated with the HDAC activity of the matching CMC (r^2=-0.79, $p<0.001$, n=32), which was tightly associated with the RNA level of IFN effector genes. Nuclear HDAC activities were also positively linked with the levels of CVL chemokine (p-values <0.05) of chemotactic function (pathway enrichment, $p<10^{-13}$). No correlation was observed with HAT activities, vaginal pH or low NO levels in CVL. Of special interests, unusually low HDAC activity, slightly high H_2O_2 level, reduced IRF-1 expression and low chemotactic chemokine level in CVL were found in a subset of Kenyan female sex workers who exhibit less susceptibility to HIV-acquisition, and have a relative quiescent baseline immunologic milieu at the vaginal mucosa.

Significance: This study provides *ex vivo* evidence to support that the soluble factors in vaginal fluid can potentially influence the regulation of immunologic genes via affecting the HDAC activities in vaginal cells. The correlation study further suggests that low HDAC activity may be the consequence of high CVL H_2O_2 level, leading to a more quiescent immunologic milieu with reduced IFN effector function and less chemotactic chemokine at baseline, an unfavorable condition for the establishment of HIV-infection. *Further investigation in how the soluble factors "shape" mucosal immunity* will enhance our understanding of the host-environment interactions.

[98] Investigating the role of ATRX in hippocampal pyramidal neurons.

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The ATRX intellectual disability gene is involved in chromatin architecture regulation, and emerging evidence shows that chromatin alterations contribute to flexibility for synaptic plasticity during learning and memory. We previously determined that ATRX regulates gene expression in the developing mouse brain via deposition of histone variant H3.3 at GC-rich genes which facilitates transcription by RNA Polymerase II. Therefore, I hypothesize that ATRX is required for synaptic plasticity in hippocampal pyramidal neurons by regulating gene expression at the epigenetic level. We mated *Atrx* floxed mice to a *CaMKII*-Cre recombinase line to create a loss-of-function mouse model in which ATRX was deleted in forebrain pyramidal neurons starting at approximately postnatal day 20 ($Atrx^{CaMKIICre}$). At three months of age these mice underwent a battery of behavioural tests. There was a significant difference between $Atrx^{CaMKIICre}$ and control mice in the time spent in centre of the open field test, and an increase in time spent in the open arms of the elevated plus maze, an indication of decreased anxiety. In the Morris water maze mutant mice could remember the target location one day following the training period. However, they displayed impaired long-term memory one week after training and decreased freezing behaviour during the contextual fear conditioning task, both spatial memory processes heavily reliant on the hippocampus. Furthermore, the touchscreen Paired-Associate Learning revealed impaired spatial learning in the $Atrx^{CaMKIICre}$ mice. Transcriptional profiling by RNA-sequencing of control and $Atrx^{CaMKIICre}$ hippocampi revealed increased expression of *Tryptophan dioxygenase 2* (*Tdo2*), a tryptophan metabolism gene responsible for converting tryptophan to kynurenine. Increased kynurenine and its downstream metabolites have been implicated in neurological diseases such as intellectual disability, Alzheimer's, and schizophrenia. Quantitative real-time PCR confirmed this increase. In addition, *in situ* hybridization using a probe for *Tdo2* indicated an increase in expression of the gene specifically in the dentate gyrus. TDO2 is the rate-limiting enzyme in the conversion of tryptophan to kynurenine, and tryptophan is also a precursor for the neurotransmitter serotonin, linked to anxiety. A serotonin ELISA performed in the hippocampus revealed increased serotonin, likely underlying the decreased anxiety observed in the $Atrx^{CaMKIICre}$ mice. More experiments are underway to evaluate morphological effects of ATRX loss in the hippocampus of $Atrx^{CaMKIICre}$ mice. Our study has identified molecular defects in the hippocampus that could potentially underlie anxiety and memory impairments in *Atrx* mutant mice.

[99] EpiSpermHis: A Galaxy Standalone sperm histone data analysis platform for optimization of sperm epigenome analysis in mice and men to be used for the identification of epigenetic signatures in sperm associated with environmental perturbation.

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Sperm has a unique chromatin conformation with the majority of somatic histones being replaced by protamines and only 1 % retained in sperm from mice and 10% from men. Thus, unlike a typical ChIP-seq profile generated from targeting a histone modification there are fewer histone peaks and these tend to be distributed over CpG (5'-C-phosphate-G-3') enriched regions [1]. Effects of the paternal environment including stress, diet and toxicants have been linked to negative outcomes for offspring including birth defects and increased risks for complex diseases. These paternal effects may occur via non- genetic inheritance, through epigenetic mechanisms including DNA methylation, post- translational modifications of histones and noncoding RNAs. We hypothesize that, the sperm epigenome in men, specifically histone H3 lysine 4 tri-methylation (H3K4me3), can influence offspring development and health. The challenges in analyzing and quantitating ChIP-seq data from sperm with currently available software is the ability to detect and quantify differences not just in peak enrichment but also the broad domains. We have developed a Standalone Docker [2] container with Galaxy [3], tools for NGS and quantitative comparison of histone modification levels in sperm. The Galaxy framework will allow biologists, with no command-line knowledge. To perform an optimal pre-processing and to address other challenges in data analysis, we have incorporated efficient bioinformatics pipelines for analyzing sperm epigenome data, by using currently available tools (Bowtie2, Trimmomatic, Picard tools, MACS2, etc.), and in-house developed tools to address the challenges of identification of the most reliable peak calling method with appropriate parameters while considering the unique chromatin configuration in sperm. All the in-house built tools or scripts will be made available through the Galaxy ToolShed [4], only after the tool passes a test. We have included several tools and Statistical analysis, R [5] packages, which would not restrict the users to the provided pipelines and they would be able to adapt it according to their research requirements. The advantage of making a Docker based Galaxy standalone workbench is that it will also guarantee reproducibility and security to the researchers.

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[100] HNF4a mediated epigenetic regulation of liver-specific genes requires its interaction with TET3.

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Transcription factors (TFs) are key components of gene regulation and their binding to *cis* regulatory regions can establish the expression status of genes, leading to the specification of cell fate. Hepatocyte nuclear factor 4 alpha (HNF4a), a liver enriched transcription factor, plays an important role in specification of hepatic progenitor cells by regulating a network of TFs that controls the onset of hepatocyte cell fate. Our HNF4a ChIP-seq, MeDIP-seq hMeDIP-seq data for hepatocytes showed that HNF4a primarily binds to enhancer regions with a very low level of DNA methylation and high hydroxymethylation. Further, we confirmed that HNF4A physically interacts with TET3 in hepatocytes and HepG2 cells to hydroxymethylate these enhancers. Knockdown of HNF4a or TET3 results in a significant decrease in global hydroxymethylation in HepG2 cells, indicating its role in epigenetically shaping enhancers by retaining transcription permissive marks at enhancers required to maintain expression of liver specific genes. Furthermore, we found that HNF4A induces TET3 expression via direct binding to its *cis*-regulatory elements. Based on these observations, we confirmed that HNF4a regulates liver specific gene expression by its interactions with TET3 DNA demethylases, to protect distal gene regulatory sites from the transcriptional repressive influences of DNA methylation during liver development. Overall, our findings present a novel mechanism of HN4a mediated genes regulation in liver.

[101] Transcriptomic variations in the developing hippocampus associate with dynamic histone variant H2A.Z deposition

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Brain development requires the precise coordination of transcriptional cascades to produce the maturing neural infrastructure. Deciphering how such transcriptomic changes are controlled is central to understanding the molecular mechanisms that drive neurodevelopment. Epigenetic mechanisms provide a plausible regulatory candidate. Variations in the deposition of the histone variant H2A.Z in relation to transcriptomic changes were investigated as a possible epigenetic regulator of early hippocampal development in the rat between postnatal day 4 (P4) and P21. Histone variants are specialized chromatin components that have unique and non-redundant functions in maintaining chromosomal integrity. Distinct transcriptomic profiles were deduced at P4 and P21 using RNA-sequencing. Upregulated expression at P4 was related to neurogenesis and cell cycle control. Upregulated P21 transcription associated with ion transport and synaptogenesis. Parallel ChIP-sequencing demonstrated that H2A.Z is dynamically incorporated *in situ* during postnatal neurodevelopment. H2A.Z was remodeled genome-wide and variations at transcription start sites correlated with alterations in development-dependent transcription. Ontological analyses of the overlapping H2A.Z peaks and transcripts at P4 emphasize the importance of the developing neural infrastructure (neurogenesis, cell cycle control), and at P21, the refinement of synaptic circuitry (neurotransmission, synaptogenesis). This work demonstrates that genome-wide, postnatal rearrangements of H2A.Z in the mammalian brain associate with neurodevelopment. H2A.Z variations are likely part of a broader epigenetic regulation of neurodevelopmental transcription.

[102] The MCM2 Interactome and the Mechanism of the CMG Helicase Activity over Nucleosomes

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Eukaryotic DNA replication is a highly disruptive process where efficient unwinding of double-stranded DNA by the replisome is contingent on brisk disassembly and reassembly of nucleosomes at replication forks. Newly synthesized DNA found behind the replication fork is quickly repackaged with an equal population of old and new histones. New histones are synthesized to prevent nucleosome dilution during DNA replication. Old parental histones carry a wide array of post-translational modifications (PTMs), which are believed to help nucleate the addition of equivalent marks on new naïve histones. These PTMs are not encoded in our genome and therefore, are epigenetically inherited. While histone PTM signatures can affect downstream expression pathways and chromatin compaction, the mechanism by which parental histones are segregated at replication forks remains poorly understood.

Recent work has identified an N-terminal histone binding domain (HBD) unique to the minichromosome maintenance 2 (MCM2) protein subunit of the CDC45/MCM2-7/GINS (CMG) DNA helicase, which acts at the forefront of replication forks. Crystallography analysis of the MCM2 HBD shows that it seems to replace the DNA interactions that would be lost upon histone eviction during replication. Moreover, MCM2 binding properties indicates it has a sharp bias for binding parental histones over naïve histones, unlike other proteins that have been previously implicated. However, MCM2-histone interaction does not occur alone, it often requires the concerted help of other, unidentified, factors. We believe identification of MCM2 interactors will help discern the mechanism behind parental histone deposition.

To establish the MCM2 interactome, I am performing proximity-based labelling (BioID) of proteins interacting with wildtype MCM2 and an MCM2 HBD deletion mutant. Proteins uniquely interacting with the MCM2 HBD will be titrated across an *in vitro* CMG helicase assay acting on chromatinized DNA, which normally stalls in the presence of nucleosomes. Therefore, proteins with histone eviction capacity, upon titration to our helicase assay, are likely to help the CMG yield single-stranded DNA. These identified proteins may further play critical roles in the inheritance of epigenetic information.

[105] Consequence of SWI/SNF complex dysregulation in the Malignant Rhabdoid Tumor and the Ovarian Clear Cell Carcinoma

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Genetic mutations in SWI/SNF subunits are recurrent events in cancer and are thought to contribute to carcinogenesis. Cancer subtypes show remarkable specificity to SWI/SNF subunit loss (e.g. SMARCB1 in malignant rhabdoid tumour (MRT); ARID1A in ovarian clear cell carcinoma (OCCC); SMARCA4 in small cell carcinoma of the ovary, hypercalcemic type (SCCOHT); SSX-SS18 fusion in synovial sarcoma), while other SWI/SNF mutant cancers can be driven by loss of any of multiple subunits, such as dedifferentiated endometrial carcinoma which can be driven by loss of ARID1a/1b; SMARCA4 or SMARCB1. The SWI/SNF complex plays important roles in transcriptional regulation but the drivers of selection for specific SWI/SNF subunit disruption in cancer subtypes is currently unknown. To investigate the role of *SMARCB1* and *ARID1A* in MRT and OCCC, we performed ATAC-seq, native ChIP-seq and RNA-seq on MRT and OCCC isogenic cell lines models: a MRT cell line engineered to re-express *SMARCB1*, and an OCCC line with biallelic loss of *ARID1A*. We obtained datasets for ATAC-seq, ChIP-seq (H3K4me3, H3K4me1, H3K27ac, H3K27me3, H3K36me3, H3K9me3), and RNA-seq. We identified 3269 and 2398 open chromatin regions in the presence and absence of SMARCB1 respectively, of which 1624 (49.7%) were found in the presence of SMARCB1. Moreover, we identified directionality in the number of active enhancers in the presence of SMARCB1. FOXO1 and SMAD3 binding sites were statistically enriched in the SMARCB1 specific enhancers and genes up-regulated in SMARCB1+ cells are implicated in cell morphogenesis and differentiation. In contrast, directionality was not observed with ARID1A loss in the isogeneic OCCC lines with 9562 (27.0% of total) and 9381 (26.8% of total) unique open chromatin regions in the presence and absence of ARID1A respectively. ARID1A dependent active enhancers showed significant enrichment for FRA-1 and AP-1 binding sites and downregulation of genes related to cell adhesion, urogenital system development, and NF-kappa B signaling pathways. Our analysis revealed that loss of expression of the SWI/SNF subunits SMARCB1 and ARID1A is associated with subunit specific genome-wide chromatin dysfunction leading to distinct enhancer alterations and differential expression of genes.

[106] Ancient enhancers share conserved gene regulatory activity during early vertebrate heart development

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In contrast to the transcription factors that control heart development, heart enhancers show weak phylogenetic constraint. However less is known about the identity of, and evolutionary constraints on enhancers active in lineages leading up to the formation of the heart. To identify heart enhancers active during the initial stages of heart development, we labelled and isolated cardiac progenitor cells from zebrafish embryos using a mouse enhancer previously shown to label cardiac cells during gastrulation. Intra-embryo comparisons using RNA-seq and ATAC-seq, together with lineage tracing and RNA *in situ* hybridization, confirmed that we obtained a population of cells enriched for cardiac progenitors. Inter-species comparisons of open chromatin regions using direct and indirect alignment methods revealed ~170 conserved non-coding elements that mapped to open chromatin in the orthologous human regions. Using *in vivo* reporter gene assays, we found that the majority of zebrafish epigenetically conserved non-coding elements (eCNEs) drove expression in the heart, and that orthologous human and zebrafish eCNEs could recapitulate spatial temporal expression patterns during zebrafish development despite sharing modest sequence identity. Globally we found ~6000 eCNEs shared between zebrafish and humans that were collectively enriched for polycomb repressive complex 2 binding and homeodomain proteins. Overall this study adds a set of ancient enhancers to the lexicon of regulatory elements active during vertebrate heart development and suggests that eCNEs can direct diverse spatial-temporal gene expression patterns during vertebrate development.

[107] Loss of SMARCA4 and CTCF as biomarkers for anti-cancer therapy

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Our overarching goal is to identify deregulated epigenetic mechanisms that contribute to the progression of breast and ovarian cancer, and to use this information to identify new therapeutic interventions. Here, we report that loss of SMARCA4 acts as a biomarker predicting the efficacy of bromodomain inhibitors (BETi) and report that deletion of a single allele of the epigenetic regulatory protein CTCF acts as a predictive biomarker for cisplatin activity.

The antitumor activity of BETi has been demonstrated across numerous types of cancer. As such, these inhibitors are currently undergoing widespread clinical evaluation. However, predictive biomarkers allowing the stratification of tumors into responders and non-responders to BETi are lacking. Now, we report significant anti-proliferative effects of BETi *in vitro* and *in vivo* against aggressive ovarian and lung cancer models lacking the SWI/SNF-related, SMARCA4 protein. Importantly, our *in vivo* experiments, using an orthotopic model of ovarian cancer, employed BETi at concentrations considerably lower than have been previously proven effective against solid tumors. Restoration of SMARCA4 promoted resistance to BETi in these models, and conversely, knockdown of SMARCA4 sensitized resistant cells to BETi. Transcriptomic analysis revealed that exposure to BETi potently down-regulated the oncogenic receptor tyrosine kinase HER3 in SMARCA4-deficient cells. Repression of this pathway is found to be an important determinant of response to BETi in cells harboring a loss of SMARCA4. Overall, we propose that BETi represent a rational therapeutic strategy in poor prognosis, SMARCA4 deficient cancers.

CTCF is a multifunctional epigenetic-regulatory protein. Mice heterozygous for CTCF reveal that CTCF is a *bona fide* tumor suppressor gene. Consistent with this, CTCF copy number loss is observed in a spectrum of cancers including ~50% of breast tumors. However, a role for CTCF deletion in breast cancer development or progression has not been explored. Using CRISPR/Cas9, we deleted a single copy of CTCF in mammary epithelial cells (MCF10A). Using this system, we discovered for the first time that CTCF plays a role in the repair of double strand breaks through the homologous recombination repair pathway (Science Advances, 2017). Here, CTCF binds BRCA2 and facilitates its recruitment to breakages, dependent on CTCF post-translational modification by poly (ADP-ribosylation). Importantly, we now show that cells with a loss of CTCF are acutely sensitive to the DNA damaging agent cisplatin. Further, we present, for the first time, that loss of CTCF activates a transcriptional network that allows normal cells to acquire pro-invasive properties. Overall, we identify a new role for CTCF in preventing cell invasion, and identify the loss of CTCF as an *Achilles heel* for cisplatin treatment.

[108] SMCHD1 is required for normal expansion of muscle progenitor cell population

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Facioscapulohumeral muscular dystrophy (FSHD) is a neuromuscular disease-causing weakness of the facial, shoulder and upper arm muscles. FSHD1 is associated with deletions of the D4Z4 tandem repeat DNA fragments on chromosome 4q35, leading to a decrease in epigenetic repression of the DUX4 gene within the locus. In the absence of a truncated D4Z4 locus, mutations in the SMCHD1 gene have also been shown to cause the onset of FSHD (FSHD2), as this protein appears to be necessary for epigenetic silencing of the disease-causing genes. Importantly, altered SMCHD1 function contributes to the manifestation of both forms of FSHD, since mutations in SMCHD1 are known to increase the disease severity of FSHD1. However, we continue to lack an understanding of how SMCHD1 contributes to epigenetic silencing of FSHD causing genes in healthy muscle.

To characterize the mechanism by which SMCHD1 contributes to disease gene silencing, we first needed to identify the stage of regeneration in which muscle defects could be observed. For this we performed knocked-down of SMCHD1 in immortalized myoblasts from FSHD1 patients and healthy controls. Using a Doxycycline-inducible shRNA expression system, we observed that loss of SMCHD1 did not result in any gross morphological changes in either myoblast population. However, measuring the growth rate of the myoblasts after SMCHD1 knockdown, we observed that the number of proliferating progenitor cells decreased by 50% after a period of 7 days. To determine whether the decrease in progenitor numbers was due decreased proliferation, we performed an EdU (5-ethynyl-2´-deoxyuridine) pulse labeling experiment. Staining for EdU incorporation, we observed that a 50% decrease in the number of cells that had traversed S-phase for the SMCHD1 knockdown population. This decreased proliferation strongly co-relates with our cell counting data, indicating that SMCHD1 is required for proliferation of the muscle progenitor population. In contrast, myogenic differentiation assays showed no defect in myotubes formation when SMCHD1 was knocked down. Based on these early studies, we have begun to examine how SMCHD1 contributes to gene silencing to allow efficient expansion of the muscle progenitor population.

[109] Preconception urinary phthalate concentrations and sperm DNA methylation profiles among men undergoing IVF treatment

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BACKGROUND: Several phthalate compounds and their metabolites are known endocrine disrupting compounds and are pervasive environmental contaminants. Several epidemiologic studies have associated select urinary phthalate metabolites with poor semen parameters and other adverse reproductive outcomes. Rodent studies report that prenatal phthalate exposures induced sperm DMRs in the offspring, but the influence of adult preconception phthalate exposure on sperm DNA methylation in humans is unknown.

OBJECTIVE: To examine the potential associations between phthalate and phthalate replacements exposure and sperm DNA methylation among men undergoing in-vitro fertilization.

METHODS: The first 48 couples from the Sperm Environmental Epigenetics and Development Study (SEEDS) provided a spot urine sample on the same day as semen sample procurement. Sperm DNA methylation was assessed with the HumanMethylation 450K array. Seventeen urinary phthalate and 1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) metabolite concentrations were measured from spot urine samples. After correction of DNA methylation measurements for background levels, probe bias, and batch effects, the A-clust algorithm was employed to identify co-regulated regions among the remaining CpG sites. Differentially methylated regions (DMRs) associated with urinary metabolite concentrations were identified via linear models, corrected for false-discovery rate.

RESULTS: Adjusting for age, BMI, and current smoking, 131 DMRs were associated with at least one urinary metabolite. Most sperm DMRs were associated with anti-androgenic metabolites, including mono(2-ethylhexyl) phthalate (MEHP, n=83), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP, n=16), mono-n-butyl phthalate (MBP, n=22), and cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl (MCOCH, n=7). The DMRs were enriched in lincRNAs as well as in regions near coding regions. Functional analyses of DMRs revealed enrichment of genes related to growth and development as well as cellular function and maintenance. Finally, 13% of sperm DMRs were inversely associated with high quality blastocyst-stage embryos after IVF.

DISCUSSION: To our knowledge, this is the first study to report that preconception urinary phthalate metabolite concentrations are associated with sperm DNA methylation in humans. These results suggest that paternal adult environmental conditions may influence epigenetic reprogramming during spermatogenesis, and in turn, influence early-life development.

[110] Cfp1/Cps40 stabilizes MLL complex formation through multi-valent interactions

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The SET1 family of methyltransferases (SET1A/B, MLL1-4) are responsible for the methylation of Lys-4 on histone H3 (H3K4), a post-translational modification that is predominantly localized in the promoter and enhancer regions of actively transcribed genes. Moreover, mis-regulation or mutations of members of SET1 family of PKMTs is now recognized as a hallmark of cancers. Members of the SET1 family of methyltransferases require several regulatory subunits to efficiently methylate histone H3. These subunits include DPY30/Cps25, WDR82/Cps30, WDR5/Cps35, Cfp1/Cps40, RbBP5/Cps50 and Ash2L/Cps60. Once bound to SET1, these proteins form a protein complex referred to as COMPlex ASsociated to SET1 (COMPASS) whose role is to fully activate the methyltransferase activity of SET1. While previous structural studies highlighted important molecular underpinnings underlying the interaction of COMPASS subunits1–3, they failed to capture the overall architecture of COMPASS. In addition, owing to the low resolution of a Cryo-EM structure of COMPASS4, an in-depth understanding of the structural determinants controlling the formation and regulation of COMPASS remains elusive. Here we present the purification and partial *in vitro* reconstitution of COMPASS from *Chaetomium thermophilum*. Biochemical evidence suggest that Cps40 is able to regulate the COMPASS activity through interacting with regions found at the N-terminus of SET1 catalytic domain as well RbBP5/Cps50 and WDR5/Cps35 likely prompting these subunits to shape the SET domain into an active conformation. These results pave the way for further in-depth understanding of COMPASS's structure and regulation, especially the Cfp1/Cps40's role in COMPASS biology.

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[111] Epigenetic Conversions and Links to Paused Replication Forks

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The transmission of epigenetic marks on histones and DNA is an integral part of eukaryotic DNA replication. This transmission culminates in the reconstitution of pre-existing chromatin structures or, alternatively, in an epigenetic conversion of the replicated locus.

We have developed an assay for the quantitative assessment of the frequency of epigenetic conversions at the sub-telomeres of *S. cerevisiae*. We have documented that the destruction of Chromatin Assembly Factor -1 (CAF-1) or the DNA helicase *RRM3* substantially reduce the frequency of such conversions. CAF-I is a histone chaperone, which reassembles the disassembled nucleosomes immediately after the passage of the replication forks. *RRM3* encodes a helicase that relives replication forks, which have paused at sites of tightly-bound non-histone proteins or at sites of active transcription. Of note, subtelomeric DNA contains multiple *RRM3*-dependent replication pausing sites. Current models suggest that both Rrm3p and CAF-1 are recruited to replication forks via an interaction with the Proliferating Cell Nuclear Antigen (PCNA, *POL30*) and that this interaction is regulated by the DBF4-Dependent Kinase, DDK.

Here we present our data on the frequency of epigenetic conversions in different genetic backgrounds. We will also present our recent studies on the role of two kinases (CDK and DDK) that phosphorylate CAF-I and regulate its association with chromatin and its stability. We propose a model for replication-coupled epigenetic conversions.

[112] Investigation of noncoding mutations through the lens of the primary prostate cancer epigenome reveal significantly mutated cis-regulatory elements near *FOXA1*

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Prostate cancer (PCa) is the most commonly diagnosed non-cutaneous malignancy among North American men. There remains a knowledge gap in understanding why some primary PCa tumors become aggressive and progress, whereas other tumors remain indolent. Despite the previous efforts to mine the primary PCa exome for recurrently mutated genes, only six were reported to be mutated in more than 2% of patients, including *FOXA1* that is mutated in 3-4% of primary PCa patients 1. In contrast, the other 98% of the genome containing cis-regulatory elements (CREs) and transcription factor binding sites critical for transcriptional regulation, have been largely underexplored 2. In this work, we analyzed somatic mutations called from the whole-genome sequences of 200 primary PCa patients to elucidate novel driver mutations. By overlapping these mutations to H3K27ac ChIP-seq peaks we previously conducted on primary tumor tissues, we find that 10% of all mutations map to active CREs. Through binomial testing, we identified significantly mutated elements (SMEs) in the prostate cancer genome, including three in the neighbourhood of *FOXA1*. *FOXA1* encodes a pioneer transcription factor that help facilitate androgen receptor transcriptional program in driving PCa development. Analysis of gene essentiality screen data reveal *FOXA1* to be an essential gene across PCa cell lines, which we validated through siRNA-mediated knockdown, resulting in a significant drop in PCa cell proliferation. CRISPR/Cas9-mediated deletion of the SMEs resulted in a significant decrease in *FOXA1* expression, suggesting these elements regulate *FOXA1* expression. Moreover, through the use of luciferase assays, mutations mapping to these SMEs were shown to significantly alter the transactivation potential of the elements. Noteworthy, PCa patients that harbour mutations in the SMEs did not have mutations mapping to the *FOXA1* gene, and vice versa. Overall, our work identified SMEs that regulate *FOXA1*, suggesting PCa patients with altered *FOXA1* may exceed the 3-4% identified in the gene alone, and further reinforcing FOXA1 as a therapeutic target in prostate cancer.

[113] Epigenetic reprogramming in a human T-cell acute lymphoblastic leukaemia model

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T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive blood cancer of malignantly transformed immature thymocytes. While epigenetic reprogramming has been implicated in the initiation of T-ALL the lack of an *in vitro* model and appropriate normal controls has hampered the quantitative measurements of epigenetic signatures associated with T-ALL. To address this challenge, we have performed temporal epigenetic and transcriptome profiling of a novel human T-ALL model and untransformed controls. In this model, cord blood derived CD34+ cells are transduced with a constitutively active NOTCH1 allele, TAL1, BMI1 and LMO2 and cultured *in vitro* under conditions that promote T-cell differentiation. Three time points following transfection (Day 14, 24 and 47) were collected for epigenetic and transcriptome profiling. Hierarchical clustering of protein coding gene expression, H3K27me3, or H3K4me3 promoter density separated transduced from non-transduced CD34+ cells regardless of the *in vitro* collection day. Directionality was observed in epigenetic reprogramming of transduced CD34+ cells vs. their normal comparators. H3K4me3 marked regions increased genome-wide and were enriched in ETS and RUNX binding motifs. Dynamic reprogramming was also observed in H3K27me3 with regions that lost H3K27me3 enriched in TAL1 motifs, consistent with its known role in driving an oncogenic transcriptional program. Combining epigenetic and transcriptional deregulated genes associated with T-ALL allowed for the identification of putative targets of the transduced oncogenes. The dynamic epigenetic reprogramming revealed by this study has led to new insights into the mechanisms of T-ALL initiation and has the potential to provide novel therapeutic targets in the future.

[114] An integrative roadmap to PAX3 target gene networks in melanocytes and melanoma

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Melanoma accounts for 70% of skin cancer-associated deaths, despite representing a small fraction of all skin cancers. The developmental origin of melanocytes is thought to be a key driver of this aggressive behaviour. In this context, melanocyte identity is determined during embryogenesis by the hierarchical action of transcription factors, exemplified by MITF, SOX10 and PAX3. Each of these proteins has key roles in melanoma, reflecting their capacity to control pathogenic gene expression programs. Within this scheme, PAX3 controls cell division and differentiation by recognizing specific target sequences in the genome and altering expression of associated genes. To this end, PAX3 contains two DNA-binding domains, the homeodomain and the paired domain, which itself comprises two subdomains. In theory, the inherent modularity in this architecture permits the recognition of multiple DNA-binding patterns, yet we have not had a comprehensive view of PAX3 DNA-binding specificity or associated targets. *We hypothesize that PAX3 utilizes multiple modes of DNA recognition that can contribute to distinct functional outputs during melanoma progression.* To address this, we have repurposed data derived from cyclic amplification and selection of targets to statistically model DNA-binding specificities for human and mouse PAX3 proteins. Significantly, these profiles represent the first set of optimal motifs described for full-length PAX3. The robustness of this library was validated *in situ* by calculating its enrichment across published ChIP-seq datasets for exogenous PAX3 and the PAX3-FOXO1 pathogenic variant in muscle. This provided a foundation for predicting PAX3 occupancy in putative cell-specific regulatory regions defined using epigenomic signatures. Notably, PAX3 motifs were significantly enriched in human melanocyte enhancer regions derived from the Roadmap Epigenomics Project, as well as mouse melanocyte enhancers demarcated by H3K4me1 and adjacent EP300 occupancy. To identify downstream targets of PAX3, we used RNA-sequencing to profile differential gene expression following PAX3 attenuation across syngeneic melanocyte and melanoma cell lines. Putative targets were subsequently integrated with predicted PAX3 occupancy to connect distinct DNA-binding profiles to transcriptional pathways across cell types. Collectively, these analyses provide novel insight into the discrete target gene networks associated with the differential use of PAX3 DNA-binding modules and how these programs may be altered during progression from untransformed melanocyte to metastatic melanoma. In addition, they provide a framework for defining PAX3 regulatory networks across a broad range of PAX3-expressing cell types.

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