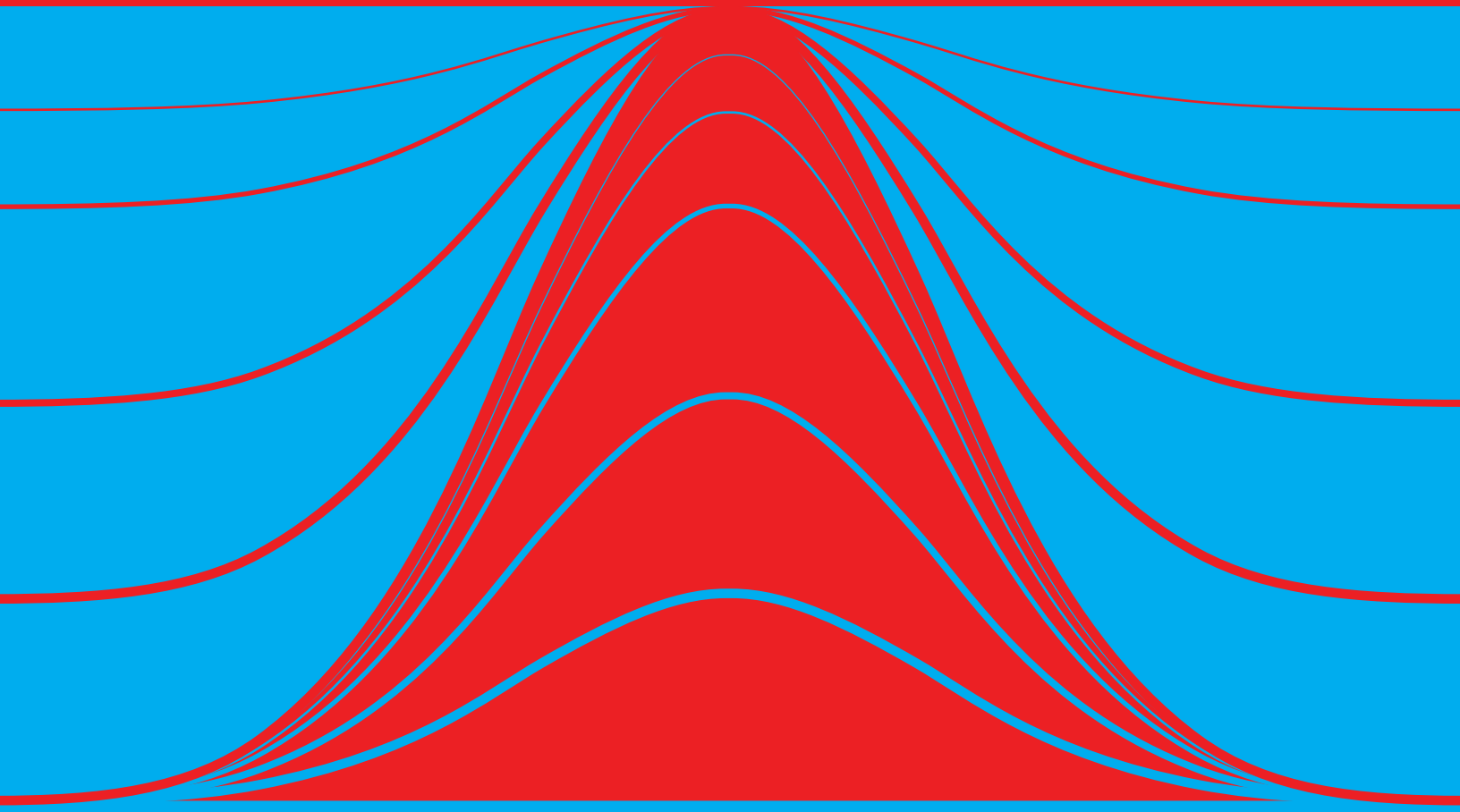
CEEHRC NETWORK
Canadian Epigenetics, Environment and Health Research Consortium Network

THE 5TH CANADIAN CONFERENCE ON

EPIGENETICS

30 September – 3 October 2018

Estérel Resort, Estérel, Québec, Canada



MEETING PROGRAM

EPIGENETICS CANADA

GenomeCanada

CIHR IRSC

ESTĒREL RESORT

Welcome!

Dear Colleagues,

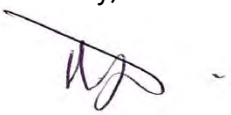
It is a pleasure to welcome you to Estérel Resort for the 5th Canadian Conference on Epigenetics.

This year is our largest meeting to date bringing together over 200 Canadian epigenetics researchers, along with key international leaders in the field, to engage in cross-disciplinary dialogue on the state of epigenetics research in Canada and beyond. The scientific program includes distinguished invited speakers in the fields of epigenetics and epigenomics covering topics including cancer epigenetics, neuroepigenetics, chromatin organization, epigenetic regulation in the immune system and during development, and new developments and advancements in epigenomics technology. These topics will be covered over 9 sessions that include plenary lectures and short oral presentations, a computational epigenetics workshop, and 2 poster sessions. We hope that the meeting format will encourage interaction and networking between young scientists and Principal Investigators while providing an atmosphere that stimulates scientific debate and catalyzes new collaborations.

This year continues our tradition of organizing this meeting as a collaboration between the Canadian Epigenetics, Environment and Health Research Consortium (CEEHRC) Network and the "Epigenetics, EH!" conference series. The aim of the CEEHRC Network is to connect Canadian epigenetics researchers and expand their reach to the broader health research community in Canada and beyond. The CEEHRC Network also facilitates access to state-of-the-science epigenomic mapping capacity and curated reference human epigenomic datasets; produces and curates epigenetic and epigenomics tools, software and protocols; and curates Canadian jobs, training opportunities, and events relevant to the epigenetics community. 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 CEEHRC Network more broadly.

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

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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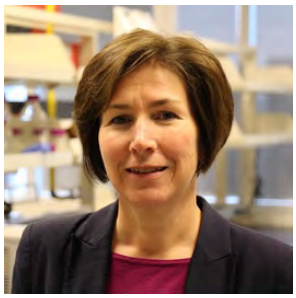
Organizing Committee



Cheryl Arrowsmith
University of Toronto

Nathalie Bérubé
Western University

Steve Bilodeau
Université Laval

Guillaume Bourque
McGill University

Marjorie Brand
Ottawa Hospital Research Institute

Carolyn Brown
University of British Columbia

Steven Jones
BC Cancer

Martin Hirst
University of British Columbia

Sarah Kimmins
McGill University

Serge McGraw
Université de Montréal

Sinéad Aherne
BC Cancer

Tony Kwan
McGill University

Keynote Speaker

Cigall Kadoch

Assistant Professor, Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Broad Institute

Dr. Kadoch established her independent laboratory in 2014, at age 28, one of the youngest scientists ever appointed to the Harvard Medical School faculty, immediately following completion of her Ph.D. studies in Cancer Biology at Stanford University working with developmental biologist Gerald Crabtree. She has quickly become a leading expert in chromatin and gene regulation and is internationally recognized for her groundbreaking studies in these areas. Specifically, her laboratory studies the structure and function of chromatin remodeling complexes such as the mammalian SWI/SNF (or BAF) complex, with emphasis on defining the mechanisms underlying cancer-specific perturbations. Of note, the recent surge in exome- and genome-wide sequencing efforts has unmasked the major, previously unappreciated contribution of these regulators to malignancy: indeed, the genes encoding subunits of mammalian SWI/SNF complexes are mutated in over 25% of human cancers.

In addition to receiving numerous prestigious awards and research grants to support her academic laboratory at Harvard, including the NIH Director's New Innovator Award, the Pew Scholar Award, and the American Cancer Society Research Scholar Award, she was named on the Forbes 2014 30 Under 30 list, MIT Technology Review 35 Innovators Under 35, Popular Science Brilliant 10 of 2016 and most recently, Business Insider's Top 30 Young Leaders in Biopharma.

Invited Speakers

Luis Barreiro

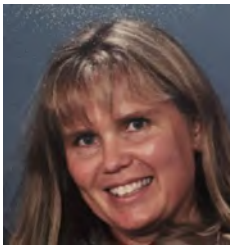
Associate Professor, Genetics Division, University of Chicago

Luis Barreiro received his undergraduate and Master's degrees in Biotechnology at Lusófona University, Lisbon, Portugal. In 2008, he obtained his PhD in Human Population Genetics from the University of Paris VII, followed by a post-doc in the Department of Human Genetics at the University of Chicago. From 2011 to 2018 he was Professor at the Université de Montréal, and a researcher at the CHU Sainte-Justine. He is currently an Associate Professor in the Genetics division, at the University of Chicago. His work has been pioneering at using population genomic data to characterize the genetic basis of inter-individual differences in immune response to infectious agents.

Annie Ciernia Vogel

Assistant Professor, Department of Biochemistry and Molecular Biology, Center for Brain Health

Annie Vogel Ciernia obtained undergraduate degrees from North Dakota State University in Biotechnology and Psychology. She then completed her PhD work with Dr. Marcelo Wood at the University of California, Irvine and postdoctoral training with Dr. Janine LaSalle and Dr. Jacqueline Crawley as an Autism Research Training Fellow the University of California, Davis. Dr. Vogel Ciernia's current research combines her interests in epigenomic regulation, neural-immune interactions, and animal behavior to study gene regulatory mechanisms in brain development and neurodevelopmental disorders.



Marie Classon

Dr. Classon has extensive experience in cancer biology in both academia and industry. Her research in recent years has been focused on evaluating mechanisms underlying epigenetically mediated cancer cell drug tolerance.

Josée Dostie

Associate Professor, Department of Biochemistry and Rosalind & Morris Goodman Cancer Research Centre

Dr. Josée Dostie is an Associate Professor in the Department of Biochemistry at McGill. She is also an associate member of the Goodman Cancer Research Center. Dr. Dostie received her Ph.D. degree in 2000 from the Biochemistry department at McGill. She completed post-doctoral training at the University of Pennsylvania (2004) and the University of Massachusetts (2007). Her research program aims to understand the relationship between three-dimensional genome organization, epigenomics, and transcription activity.

Susan Fisher

Professor, University of California, San Francisco

Susan Fisher, PhD, is a Professor in the Dept. of Obstetrics, Gynecology and Reproductive Sciences, School of Medicine, University of California, San Francisco. She is jointly appointed in the Dept. of Anatomy. Dr. Fisher is the Director of the UCSF Human Embryonic Stem Cell Program and Faculty Director of the Sandler-Moore Mass Spectrometry Core Facility. She and her group study human embryonic development and the biology/immunology of the human placenta in normal pregnancy and in pathological situations, such as preeclampsia and preterm birth. They also use mass spectrometry-based approaches for comparative proteomic analyses, including post-translational modifications.

Nicole Francis

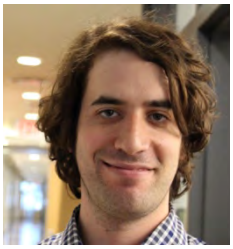
Associate Research Professor, Dept of Biochemsitry and Molecular Medicine, Institut de recherches cliniques de Montréal (IRCM) and Université de Montréal

Nicole Francis is an Associate Research Professor at the Montreal Clinical Research Institute (IRCM) and in the Biochemistry department at the Université de Montréal, and the director of the Biochemistry of Epigenetic Inheritance research unit at the IRCM. The Francis lab is interested in how chromatin-based information can be propagated through DNA replication and mitosis and uses predominantly in vitro reconstitution approaches to understand these questions.

Tamara Franklin

Assistant Professor, Department of Psychology & Neuroscience, Dalhousie University

Dr. Franklin was a post-doc in the lab of Dr. Cornelius Gross (European Molecular Biology Laboratory) and she obtained her Ph.D. from the Swiss Federal Institute of Technology (ETH-Z) under the supervision of Dr. Isabelle Mansuy. She has recently returned to Canada as an Assistant Professor at Dalhousie University. Her lab is interested in the brain function required to drive normal social interactions with a particular interest in how epigenetic regulation affects social-related neural circuits.



Michael Hoffman

Scientist, Princess Margaret Cancer Centre

Michael Hoffman creates predictive computational models to understand interactions between genome, epigenome, and phenotype in human cancers. He implemented the genome annotation method Segway, which simplifies interpretation of large multivariate genomic datasets, and was a linchpin of the NIH ENCODE Project analysis. He is a principal investigator at the Princess Margaret Cancer Centre and Assistant Professor in the Departments of Medical Biophysics and Computer Science, University of Toronto. He was named a CIHR New Investigator and has received several awards for his academic work, including the NIH K99/R00 Pathway to Independence Award, and the Ontario Early Researcher Award.

Pamela Hoodless

Professor, Department of Medical Genetics and the School of Biomedical Engineering, University of British Columbia

Dr. Hoodless completed her PhD at Queen's University at Kingston Ontario, followed by postdoctoral work at Rockefeller University in New York and The Hospital for Sick Children in Toronto. Dr. Hoodless' early work focused on gene regulation in the developing chick and mouse liver. She subsequently explored the pathways of TGFβ signal transduction and uncovered the mechanisms of Smad-mediated signalling. Her current research combines her interests in gene regulation, signalling mechanisms and embryology to explore how transcriptional networks regulate liver and heart valve formation.

Jamie Kramer

Assistant Professor, Physiology & Pharmacology, Biology, Westerm University

Jamie Kramer is a Canada Research Chair in Neuroepigenetics. His lab studies the role of epigenetics and chromatin in regulation of learning and memory. Their focus is on histone modification and chromatin remodeling complexes that are involved in neurodevelopmental disorders like intellectual disability and autism. Using a multidisciplinary approach, combining Drosophila behaviour and sub-brain transcriptome analysis, they hope to unravel genomic mechanisms involved in neuron development and function.

Anshul Kundaje

Assistant Professor, Genetics, Computer Science, Stanford University

Anshul Kundaje is an Assistant Professor of Genetics and Computer Science at Stanford University. The Kundaje lab develops statistical and machine learning methods for large-scale integrative analysis of functional genomic data to decode regulatory elements and pathways across diverse cell types and tissues and understand their role in cellular function and disease. Anshul completed his PhD in Computer Science in 2008 from Columbia University. As a PDF at Stanford University from 2008-12 and a research scientist at MIT/Broad Institute from 2012-14, he led the integrative analysis efforts for two of the largest functional genomics consortia - The Encyclopedia of DNA Elements (ENCODE) and The Roadmap Epigenomics Project

Julie Lessard

Principal Investigator, Laboratory of Chromatin Structure and Stem Cell Biology, IRIC - Université de Montréal

Julie Lessard and her team study the role of a new family of ATP-dependent SWI/SNF chromatin remodeling complexes in normal and leukemic hemopoiesis. Using mouse genetics, they identified subunits that are essential for hemopoietic stem cell (HSC) function and others that are required later in the hemopoietic hierarchy for the development of specific blood-cell lineages. Their research program suggests new exciting avenues for the manipulation of stem cells in the treatment of hematological illnesses and cancer.

Frédérick Antoine Mallette

Associate Professor, Department of Medicine, Université de Montréal, Maisonneuve-Rosemont Hospital Research Center

Frédérick A. Mallette obtained his Ph.D. from the Department of Biochemistry of the Université de Montréal, then completed a postdoctoral training at the Lady Davis Institute/McGill University, investigating novel functions of chromatin modifiers. Since 2012, he leads the Chromatin Structure and Cellular Senescence research unit of the Maisonneuve-Rosemont Hospital Research Center and is Associate Professor at the Dept. of Medicine of the Université de Montréal. Frédérick A. Mallette and his team are studying the role of chromatin structure and dynamics in cancer, as well as during cellular senescence, a critical tumor suppressive mechanism. Specifically, they investigate the interplay between histone demethylases, oncogenic cellular signaling and cancer-associated metabolic defects

Michael Wilson

Canada Research Chair in Comparative Genomics, Scientist, Genetics & Genome Biology Program, SickKids Research Institute

Michael D. Wilson is a Scientist at the Hospital for Sick Children and an Associate Professor in the department of Molecular Genetics at the University of Toronto (05/2012-present). He is a Canada Research Chair in Comparative Genomics and a Member of the Heart and Stroke/Richard Lewar Centre of Excellence. He leads a research group that uses genomic technologies, multi-species comparisons, bioinformatics and molecular biology to uncover gene and genome regulatory mechanisms that are relevant to developmental and disease processes. Wilson trained as a postdoctoral fellow in Duncan Odom's lab at Cancer Research UK – Cambridge Institute/ University of Cambridge. There he studied the evolution of transcription factor binding. He did his PhD in the molecular evolution and immunology group of Ben Koop at the University of Victoria.

Selected Speakers

Kasia Bieszczad

Assistant Professor, Department of Psychology, Rutgers University -New Brunswick

Kasia Bieszczad completed a Biology degree at McGill University and her PhD in Neurobiology & Behavior at the University of California Irvine. Her research aims to identify neurobiological mechanisms of learning and memory formation, with an emphasis on the cortical substrates of memory. Using combinations of techniques from electrophysiology to molecular epigenetics and behavior, she is building a multi-level understanding of how genes and molecules can guide neuroplasticity events in circuits and systems that transform experiences into lasting memories. She directs the Cortex, Learning, Epigenetics and Function (CLEF) lab.

Philippe Campeau

Clinical Assistant Professor, Department of Pediatrics, University of Montreal,

Philippe Campeau, MD is Clinical Assistant Professor, Department of Pediatrics, University of Montreal, and a clinical geneticist at the CHU Sainte-Justine. Dr. Campeau focuses his clinical and research activities on identifying skeletal dysplasia genes and modeling them in cellular and murine systems. The current focus of his laboratory is to better understand the processing and transport of GPI-anchored proteins, and to identify new therapies for low bone mass.

Denise Daley

Associate Professor, Department of Medicine, UBC

Denise Daley completed a PhD in Epidemiology and Biostatistics at Case Western Reserve University in 2003, followed by post-doctoral training at UBC. In 2008 she was awarded a Tier II Canadian Research Chair and appointed as an Assistant Professor at the University of British Columbia and promoted to Associate Professor in 2013. She has received numerous awards including a Michael Smith Foundation for Health Research Career Scholar Award (2008) and the CIHR Institute of Genetics Maud Menten Prize (2009) in recognition of her outstanding achievements. Dr. Daley's interests are in the study of complex diseases such as cancer, asthma, and heart disease, with a focus on gene-gene and gene-environment interactions.

Ashot Harutyunyan

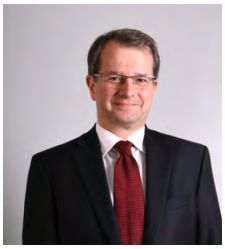
Postdoctoral Fellow, Department of Human Genetics, McGill University

Ashot Harutyunyan is a Postdoctoral Fellow in Prof. Nada Jabado's lab at McGill University. He obtained his PhD degree from Medical University of Vienna (Austria) in 2013. His postdoctoral research focuses on epigenomic changes in pediatric brain cancers due to mutations in histone H3 genes, using CRISPR/Cas9 genome editing and various high throughput sequencing techniques.

Maria Kmita

Research Unit Director, Laboratory of Genetics and Development, IRCM

Marie Kmita is a Research Professor and director of the Genetics and Development research unit at the Montreal Clinical Research Institute (IRCM). She completed post-doctoral training with Dr Denis Duboule at the University of Geneva (Switzerland). Her research program aims at understanding the transcriptional regulation of developmental genes.



Tarik Moroy

President and Scientific Director, IRCM

Tarik Möröy, PhD, is the President and Scientific Director of the Montreal Clinical Research Institute/Institut de recherches cliniques de Montréal (IRCM), as well as Director of the Hematopoiesis and Cancer research unit and Full IRCM Research Professor. He holds the Canada Research Chair (Tier 1) in Hematopoiesis and Immune Cell Differentiation. He is also Full Research Professor at the Université de Montréal and adjunct professor at McGill University. He presently leads the two major initiatives of the IRCM Strategic Plan on the Research Centre on Rare and Genetic Diseases in Adults and the establishment of a pipeline for the production of biomarkers. These actions centred around the desire to “provide passionate researchers with the ideal conditions to experiment with their boldest ideas, test their theories and pave their own way for the benefit of us all”

Theodore Perkins

Senior Scientist, Ottawa Hospital Research Institute

Theodore J. Perkins is a Senior Scientist at the Ottawa Hospital Research Institute, and an Associate Professor in the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa. His research focuses on Bioinformatics and Machine Learning, with a particular emphasis on high-throughput data analysis in the contexts of gene regulation, stem cells, and cancer.

Jüri Reimand

Principal Investigator, OICR and Assistant Professor, University of Toronto

Jüri Reimand is a principal investigator at the Ontario Institute for Cancer Research and Assistant Professor at the Department of Medical Biophysics, University of Toronto. The lab develops and applies integrative analysis techniques to learn about the biology and vulnerabilities of cancer using large multivariate omics datasets and molecular networks and pathways, with a particular focus on the non-coding cancer genome. Jüri received his PhD at the University of Tartu in Estonia and completed his postdoctoral training with Gary Bader at the Donnelly Centre in Toronto.

Karl Riabowol

Professor, Head of Aging and Immortalization Laboratory, University of Calgary

Karl Riabowol is Professor in the Departments of Biochemistry & Molecular Biology and Oncology at the University of Calgary. Dr. Riabowol obtained his Honors B.Sc. in Biology at SFU, his Doctorate in Biochemistry at the University of Arkansas for Medical Sciences and pursued Postdoctoral training at Cold Spring Harbor Laboratory (CSHL) before being promoted to the position of Staff Associate at CSHL. Current interests of the lab include developing a new model of canine aging and telomere dynamics in early development, and understanding the mechanisms impacted by the ING proteins during stem cell maintenance, cellular aging and the escape from aging seen in cancer cells. His group discovered the first member of the ING family of epigenetic regulators and they have made additional contributions to understanding the mechanisms by which the ING family of proteins regulate cell growth, apoptosis, senescence and stem cell character.

Kenjiro Shirane

Postdoctoral Fellow, Department of Medical Genetics, University of British Columbia

Kenjiro Shirane earned his undergraduate degree from Kyushu University and completed his PhD study in the spring of 2017 under the supervision of Dr. Hiroyuki Sasaki. He then joined the laboratory of Dr. Matthew Lorincz at University of British Columbia. His research focuses on understanding the mechanism of the interplay between DNA methylation and histone modifications in transcriptional regulation of genes in germ cells and early embryos.

Rapid Fire Talks

Amel Chaouch
PhD Student
McGill University

Supervisor
Paul Lasko

Eric Chater-Diehl
Postdoctoral Fellow
Sick Kids Research Institute

Supervisor
Rosanna Weksberg

Stephanie Chrysanthou
Postdoctoral Fellow
Albert Einstein College of Medicine

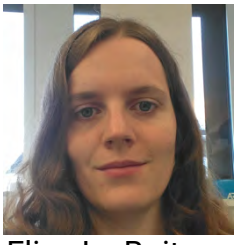
Supervisor
Meelad Dawlaty

Nergiz Dogan-Artun
Postdoctoral Fellow
University Health Network

Supervisor
Mathieu Lupien

Michael Johnston
Postdoctoral Fellow
University of Calgary

Supervisor
Marco Gallo



Elisa Le Boiteux
PhD Student
GReD

Supervisor
Philippe Arnaud

Ariane Lismer
MSc Student
McGill University

Supervisor
Sarah Kimmins

Sarah MacKinnon
PhD Student
McGill University

Supervisor
Jason Tanny

Akhil Nair
Graduate Student
University of Toronto

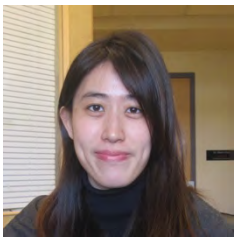
Supervisor
Art Petronis

Gurdeep Singh
PhD Student
University of Toronto

Supervisor
Jennifer Mitchell

Christophe Tav
PhD Student
Université Laval

Supervisor
Steve Bilodeau



Siyun Linda Wang
PhD Student
University of British Columbia

Supervisor
Martin Hirst

Epigenetic Technologies

Mena Farag
Sr. Sequencing Specialist
Illumina

Michael Abdo
Director of Sales - Worldwide Territories
NuGen Technologies

Brigette Brown-Kipphut
Field Application Scientist
Agilent Technologies

Carrie Ann Brown
Product Marketing Manager
Cell Signaling Technology

Francesca Meschi
Senior Scientist
10X Genomics

Program Agenda

All talks take place in Fridolin-Simard

Sunday, September 30th, 2018

15:00 - 18:30	Registration *(Reception Emotion)*
	KEYNOTE PRESENTATION
18:30 - 19:30	Cigall Kadoch (Dana-Farber Cancer Institute) *Structure and Function of Mammalian SWI/SNF Chromatin Remodeling Complexes in Human Disease*
19:30 - 21:30	Cocktail Reception *(Salon Dupuis)*

Monday, October 1st, 2018

07:00 - 08:20	Breakfast *(Bistro à Champlain)*
	Open Meeting
08:20 - 08:30	Opening Remarks Martin Hirst
	Session 1: Epigenetic Regulation During Development Chair: Serge McGraw
08:30 - 09:00	Susan Fisher (UCSF) *Genome-scale fluctuations in the cytotrophoblast epigenome over gestation and in placental pathologies*
09:00 - 09:30	Tamara Franklin (Dalhousie University) *DNA methylation signatures for Mendelian disorders of the epigenetic machinery*
09:30 - 09:45	Karl Riabowol (University of Calgary) *Maintenance of stem cell character in tumour initiating cells by the ING5 protein*
09:45 - 10:00	Kenjiro Shirane (University of British Columbia) *Role of Non-canonical H3K36me3 in Establishing Spermatogenic DNA Methylation Landscape*
10:00 - 10:30	Coffee Break *(Foyer Fridolin-Simard)*
	Session 2: Gene Regulation and Enhancers Chair: Martin Hirst
10:30 - 11:00	Pamela Hoodless (University of British Columbia) *Epigenetic mechanisms regulating enhancer switching during hepatocyte differentiation*
11:00 - 11:30	Michael Wilson (SickKids Research Institute) *Comparative epigenomics: evolution and function of cardiovascular enhancers*
	Session 3: Epigenetic Technologies Chair: Martin Hirst
11:30 - 11:42	Mena Farag (Illumina) *Illumina Technology for Epigenetics: Updates from the Field*
11:42 - 11:54	Michael Abdo (NuGEN Technologies) *Using the Power of 5-hydroxymethylcytosine (5hmC) Epigenetic Modification at Base-pair Resolution*

11:54 - 12:06	Brigette Brown-Kipphut (Agilent) *Capture your Genome in 3D*
12:06 - 12:18	Carrie Ann Brown (Cell Signaling Technolgy) *Highly Validated Antibodies for ChIP and ChIP-seq*
12:18 - 12:30	Francesca Meschi (10X Genomics) *Interrogating Chromatin Accessibility and Regulatory Landscape at Single Cell Resolution*
12:30 - 14:00	Lunch *(Bistro à Champlain)*
	Session 4: Epigenetic Dynamics in the Immune System Chair: Sarah Kimmins Agilent
14:00 - 14:30	Luis Barreiro (University of Chicago) *Epigenetic regulation of innate immune responses to infection*
14:30 - 15:00	Julie Lessard (IRIC - Université de Montréal) *Lineage-Specific Roles of the Smarcd1/2 Subunits of SWI/SNF Chromatin Remodeling Complexes in Hemopoiesis*
15:00 - 15:15	Denise Daley (University of British Columbia) *Evaluation of A Targeted Custom Capture Bisulfite Sequencing Approach*
15:15 - 15:45	Coffee Break *(Foyer Fridolin-Simard)* The Company of Biologists
	Session 5: Rapid-Fire Talks Chair: Carolyn Brown Canadian Science Publishing
15:45 - 15:50	Amel Chaouch *Expression of H3.3 oncohistones in Drosophila melanogaster alters development and compromises tissue-specific transcriptional regulation*
15:50 - 15:55	Eric Chater-Diehl *Overlapping DNA Methylation Signatures of BAF (SWI/SNF) Complex Variants Causing Nicolaides-Baraitser and Coffin-Siris Syndromes*
15:55 - 16:00	Stephanie Chrysanthou *Role of non-catalytic functions of Tet1 in embryonic stem cell state*
16:00 - 16:05	Nergiz Dogan-Artun *Identifying Critical Regulators in Triple-Negative Breast Cancer*
16:05 - 16:10	Michael Johnston *Integrative analysis of 3D genome architecture in glioblastoma self-renewing cells identifies CD276 a putative immunotherapy target*
16:10 - 16:15	Elisa Le Boiteux *Characterization of a new lncRNA candidate to (de)regulate HOX clusters in aggressive glioma*
16:15 - 16:20	Ariane Lismer *The effects of a folate deficiency on the sperm epigenome and the implications in embryo development*
16:20 - 16:25	Sarah MacKinnon *Spt5 promotes efficient transcription termination to antagonize heterochromatin*
16:25 - 16:30	Akhil Nair *Circadian DNA modification is involved in aging and complex disease*
16:30 - 16:35	Gurdeep Singh *Deciphering the enhancer sequence code of pluripotent embryonic stem cells using comparative epigenomics*

16:35 - 16:40	Christophe Tav *Towards nutritional and epigenomic interventions in prostate cancer prevention and management*
16:40 - 16:45	Siyun Linda Wang *BAF complex mutations dysregulate enhancer landscapes in malignant rhabdoid tumor and ovarian clear cell carcinoma*
18:30 - 20:00	Dinner *(Fridolin-Simard)*
Poster Session 1 (Dupuis)	
20:00 - 22:00	See Poster Assignments (page 15)

Tuesday, October 2nd, 2018

07:00 - 08:30	Breakfast *(Bistro à Champlain)*
Session 6: Chromatin Organization Chair: Cheryl Arrowsmith	
08:30 - 09:00	Josée Dostie (McGill University) *Linking RNA to chromatin landscape and organization*
09:00 - 09:30	Nicole Francis (IRCM) *A potential role for R-loops in targeting Polycomb proteins*
09:30 - 09:45	Marie Kmita (IRCM) *PRC2-dependent tissue-specific 3D chromatin architecture reveals a mechanism for the atypical contribution of PRC2 in gene activation*
09:45 - 10:00	Tarik Moroy (IRCM) *Gfi1 tethers the nucleosome-remodeling and histone-deacetylase complex to active chromatin regions in myeloid progenitors*
10:00 - 10:30	Coffee Break *(Foyer Fridolin-Simard)*
Session 7: Computational Epigenomics Chair: Guillaume Bourque	
10:30 - 11:00	Anshul Kundaje (Stanford University) *Interpretable deep learning to decode epigenomes*
11:00 - 11:30	Michael Hoffman (Princess Margaret Cancer Centre) *Virtual ChIP-seq: predicting transcription factor binding by learning from the transcriptome*
11:30 - 11:45	Theodore Perkins (Ottawa Hospital Research Institute) *RECAP reveals the true statistical significance of ChIP-seq peak calls*
11:45 - 12:00	Juri Reimand (OICR) *Candidate non-coding driver mutations in super-enhancers and long-range chromatin interaction networks across >1,800 whole cancer genomes*
12:00 - 13:30	Lunch *(Bistro à Champlain)*
Session 8: Cancer Epigenetics Chair: Steve Bilodeau	
13:30 - 14:00	Marie Classon *Epigenetic regulation of cancer cell drug tolerance*
14:00 - 14:30	Frédérick Mallette (Université de Montréal) *The role of lysine demethylases in cellular senescence and cancer*

14:30 - 14:45	Ashot Harutyunyan (McGill University) *H3K27M affects genome-wide H3K27me2 and H3K27me3 distribution and is essential for glioma tumorigenesis*
14:45 - 15:15	Coffee Break *(Foyer Fridolin-Simard)*
Bioinformatics Workshop Chairs: Martin Hirst and Guillaume Bourque Fridolin-Simard	
15:15 - 16:45	Computational Epigenomics Workshop
18:30 - 20:00	Dinner *(Fridolin-Simard)*
Poster Session 2 (Dupuis)	
20:00 - 22:00	See Poster Assignments (page 15)

Wednesday, October 3rd, 2018

07:00 - 08:30	Breakfast *(Bistro à Champlain)*
Session 9: Neuroepigenetics Chair: Nathalie Bérubé	
08:30 - 09:00	Jamie Kramer (Western University) *Chromatin regulators in learning and memory*
09:00 - 09:30	Annie Ciernia Vogel (University of British Columbia) *Epigenomic Signatures of Microglia in Autism*
09:30 - 09:45	Kasia Bieszczad (Rutgers University) *Chromatin modifications may control the precision of remembered events*
09:45 - 10:00	Philippe Campeau (Université de Montréal) *Missense mutations disrupting the helicase domain of chromatin remodeller CHD3 cause a novel neurodevelopmental syndrome with intellectual disability, macrocephaly and impaired speech and language.*
Close Meeting	
10:00 - 10:15	CEEHRC Mapping Centre updates Guillaume Bourque
10:15 - 10:30	Closing Remarks Martin Hirst
10:45	Bus departures

Poster Assignments

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7	Fiona Allum	48	Sanne Janssen	86	Emanuela Pannia
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13	Amanda Brown	58	Elisa Le Boiteux	96	Daniel Robinson
15	David Bujold	59	Seunghee Lee	98	Sarfraz Shafiq
17	Christina Castellani	61	Alice Li	100	Robert Siddaway
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23	Jennifer Chen	67	Harry MacKay	107	Gilda Stefanelli
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29	Anaïs Darracq	73	Lauren Martin	112	Kathryn Vaillancourt
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3	Antonio Ahn	45	Cynthia Horth	84	Ana Nikolic
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19	Donovan Chan	57	Felicia Lazure	101	Maruhen Amir Datsch Silveira
22	Haifen Chen	60	Lisa-Marie Legault	102	Marie-Michelle Simon
24	Sanaa Choufani	62	Fengling Li	104	Eliza Small
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35	Charles Dupras	72	Benjamin Martin	115	Jasper Wong
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40	Cristian Groza				

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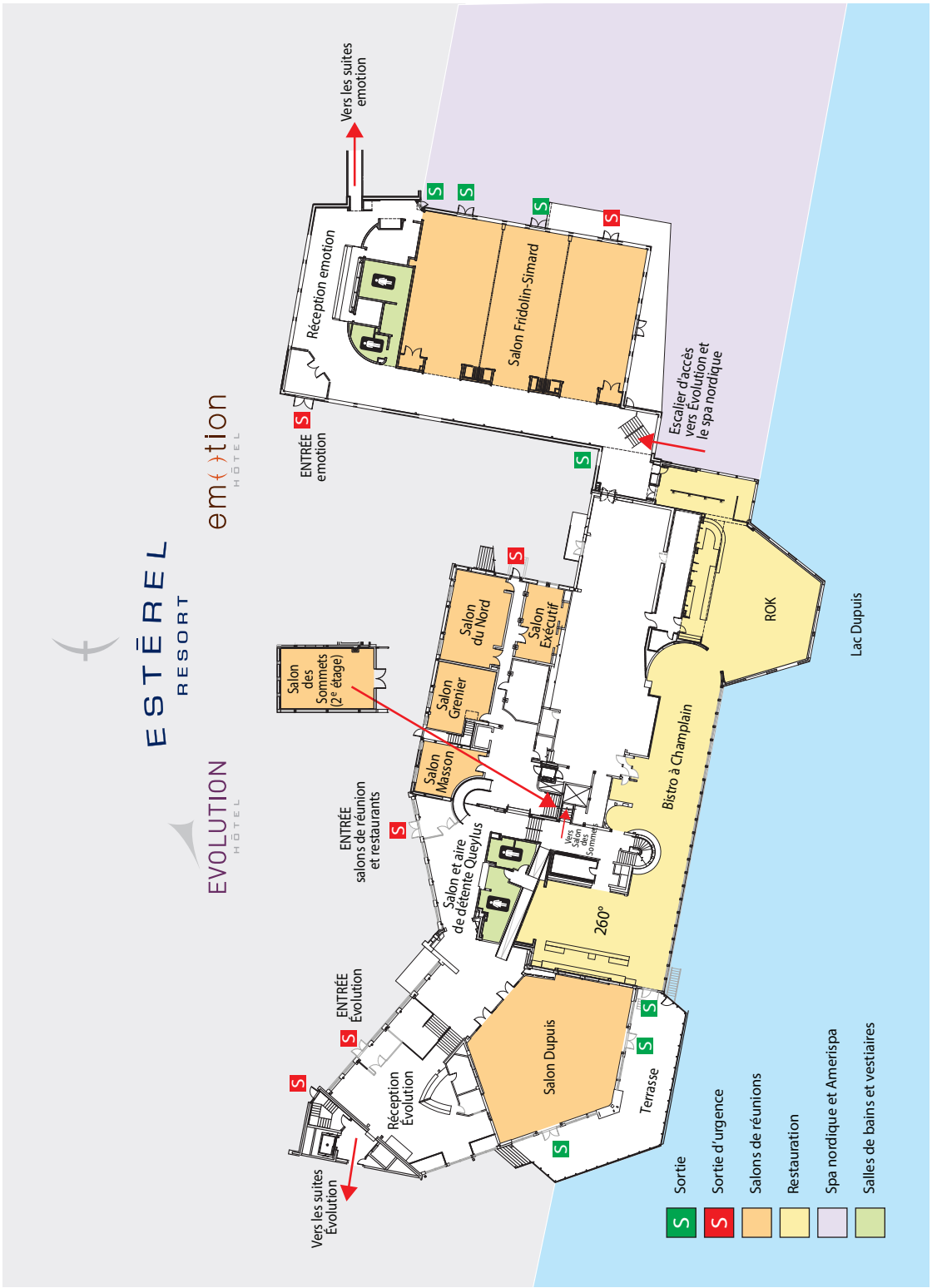

Abstracts

[1] A CRISPR/CAS9-ENGINEERED MCF-7 CELL LINE MODEL TO EVALUATE SOX2 TRANSCRIPTIONAL NETWORKS IN BREAST CANCER

Luís E. Abatti[1], Jennifer A. Mitchell[1]
[1]Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario M5S 3G5, Canada

Breast cancer is a multifactorial disease mainly characterized by aberrant levels of gene expression. The sex-determining region Y box2 (SOX2) is a key transcription factor associated with pluripotency in embryonic and reprogrammed stem cells. In mouse embryonic stem cells, Sox2 is essential for self-renewal, cooperating with a wide network of transcription factors to regulate pluripotency-associated genes. Transcriptional studies show that whereas SOX2 is highly expressed in human progenitor cells, it is normally silenced in most differentiated epithelial tissues. However, overexpression of SOX2 has been commonly observed in several forms of human tumours, including breast adenocarcinomas, where it has been linked to epithelial-mesenchymal transition (EMT), increased tumorigenesis and poorer patient prognosis. Mammary progenitor cells rely on the estrogen receptor alpha (ESR1) to differentiate and maintain an epithelial phenotype, whereas breast cancer cells are frequently undifferentiated and display estrogen resistance. My current hypothesis is that SOX2 is normally repressed by the hormonal activation of ESR1 in mammary epithelial cells. Once the estrogen pathway becomes disrupted in breast cancer cells, the repressive effect of estrogen over SOX2 expression is abolished. As a result, SOX2 expression is upregulated and the SOX2 transcription factor is able to interact with its co-factors and recruit the RNA Polymerase II complex at multiple, yet unknown genomic targets. RNA-seq analysis revealed that SOX2 displays a 600-fold upregulation ($p < 0.001$) in estrogen resistant MCF-7 cells compared to estrogen-sensitive cells, whereas short-term estrogen treatment resulted in a 50% reduction ($p < 0.001$) of the SOX2 transcript. Therefore, to better understand the regulatory network involved in SOX2 overexpression in these cells, I optimized a Cas9-mediated homology-directed repair (HDR) assay to engineer an MCF-7 cell line with a P2A-BFP fluorescent tag under the control of the endogenous SOX2 gene promoter. Although MCF-7 cells are a useful model for estrogen-sensitive breast cancer, they are less efficiently modified by CRISPR/Cas9 compared to other cell lines. Initial Cas9 HDR efforts failed to integrate this fluorescent tag into the SOX2 gene; however, I successfully increased HDR efficiency by cloning a 1500bp homology sequence surrounding the SOX2 gene into a donor plasmid flanked by two guide RNA cut sites. Once transfected into cells, this donor plasmid is cleaved by Cas9 and the homology sequence is released, resulting in higher recombination efficiency at the target gene. This system allows for the isolation of highly-expressing $SOX2^+$ MCF-7 clones and rapid analysis of trans factors contributing to SOX2 overexpression. A SOX2 functional investigation will elucidate how breast cancer cells rely on this transcription factor to maintain their tumorigenesis and how its upregulation is linked to estrogen resistance.

[2] SEASONAL AND VITAMIN D_3-MEDIATED GLOBAL EPIGENETIC REPROGRAMMING OF PERIPHERAL BLOOD CELLS AND THE IMPACT ON IMMUNE FUNCTION

Abhimanyu[1], Francois Lefebvre[4], Guillaume Bourque[4], Nina G Jablonski[5,6], Robert J Wilkinson[1,2,7,8] Anna K Coussens[1,3,9]
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Introduction: Seasonal variation in ultraviolet radiation associate with changes in serum 25(OH) vitamin D_3 levels and thus influence peripheral blood gene function. Season has been shown to effect global changes in the human transcriptome, suggesting a possible link to changes in DNA methylome. We have previously shown seasonal variation in the vitamin D status of urban South Africans (Coussens, AK et al., 2015, PNAS 112: 8052–805). Healthy young adults (18-24yrs) were reversibly 25(OH) D_3 deficient in winter as compared to summer and their serum 25(OH) D_3 status restored by six weeks high dose vitamin D_3 (50000 IU/week) in winter. In addition, the winter increase in HIV-1 replication in ex vivo infected peripheral blood mononuclear cells (PBMC) from the same participants was attenuated by 6 weeks of vitamin D_3 supplementation.
Methodology: Using stored samples from the same longitudinal cohort, we investigated the effect of variation in ultraviolet radiation and vitamin D_3 supplementation on the DNA methylation profiles of PBMC. DNA extracted from PBMC from blood (n=30 participants) taken in summer, winter and in winter following vitamin D_3 supplementation were pooled, with n=6 participants per pool. The pools were matched by 25(OH) D_3 levels, age and sex, resulting in n=5 pools per study point. To interrogate which cell type contributed to the signal, DNA was isolated from separated cells (CD14, CD8, CD3, CD19) from PBMC of n=8 participants and pooled per cell type. Pooled DNA was probed using an Infinium MethylationEPIC 850K array (Illumina Inc., USA), which uses hybridization-based fluorescence detection to investigate methylation of CpGs in islands, open chromatin, transcription factor binding sites, and enhancers. Methylation data obtained was normalized and processed using the minfi package in R.
Results: We identified a subtle impact of season and six-week high dose vitamin D_3 supplementation on methylation profiles of PBMC globally. Compared to winter we identified about 4000 differentially methylated CpG sites or DMPs using a liberal q-value cut-off ($q<0.25$) and a total of 203 DMPs for stringent F-test based comparison($q<0.05$). We identified about 300 DMPs in an overall ANOVA comparison ($q<0.15$) among the three groups. Methylation changes were largely in the gene body >intergenic > TSS> 5'UTR> 3'UTR> Exons respectively. Vitamin D_3 supplementation reversed winter associated methylation change most of DMPs in the coenzyme Q methyl transferase (COQ5) , [Summer vs Winter {$p = 2.6X10^{-9}$, $q = 0.002$}; D_3 vs Winter {$p =$

6.9X10^{-9}, q = 0.005}] and phospholipase activating enzyme A2 (PLAA), [Summer vs Winter {p = 1.5X10-^{7}, q = 0.05}; D_3 Vs Winter {p= 3.8 X10^{-7}, q= 0.09 }] genes. Both of these DMPs were less (hypo) methylated in summer and more (hyper) methylated in winter and reverted to summer hypomethylation levels by D_3 supplementation. These DMPs obtained from the pools are being validated by pyrosequencing of individual samples and their impact on regulation of HIV replication is being investigated. The upregulation of both COQ5 and PLAA as a result of hypomethylation can have effect on immune regulation observed control of HIV-1 in D_3 supplemented individuals.
Conclusions: According to our results, global methylation of PBMC DNA is subtly impacted by variation in ultraviolet radiation and high dose vitamin D_3 can affect immune function by regulating the methylation levels of COQ5 and PLAA.

[3] MARKED GLOBAL DNA HYPOMETHYLATION IS ASSOCIATED WITH CONSTITUTIVE PD-L1 EXPRESSION IN MELANOMA

Ahn, A.[1], Rodger, E.J.[1,2], Stockwell P.A.[1], Parry M.,[3] Motwani J.[1], Gallagher S.J.[4], Shklovskaya E.[4], Tiffen J.[4], Hersey P.[4], Chatterjee, A.[1,2] † Eccles, M.R.[1,2] †
[1]Department of Pathology, University of Otago, Dunedin, NZ. [2]Maurice Wilkins Centre, NZ. [3]Department of Mathematics & Statistics, University of Otago. [4]The Centenary Institute, University of Sydney, †Joint senior authorship

Expression of the PD-L1 immune checkpoint protein is used by many cancers to suppress the anti-tumor immune response. Several mechanisms that regulate PD-L1 levels have been uncovered, although whether DNA methylation plays a role in PD-L1 regulation remains unclear. In this research we have shown that marked loss of global DNA methylation, particularly in intergenic regions and repeat elements, is associated with constitutive expression of PD-L1 in melanoma cell lines compared to cell lines without PD-L1 expression. Hypomethylation and constitutive PD-L1 expression were associated with reduced levels of the de novo epigenetic regulator, DNMT3A. Additionally, genome-wide transcription levels, including immune response genes, were significantly upregulated in constitutive PD-L1 expressing cell lines, as well as in lymphocyte negative, PD-L1 positive melanomas (equivalent to PD-L1 constitutive), versus PDL1 negative melanomas in The Cancer Genome Atlas database (TCGA). Finally, decitabine-mediated inhibition of global methylation in melanoma cells led to increased PD-L1 and immune response gene expression. Therefore, in summary, using integrated genomic analysis we identified that global DNA methylation influences PD-L1 expression in melanoma, and hence the ability of melanoma cells to evade anti-tumor immune responses. In my poster I will discuss these as well as additional findings made in this project. These results have implications for combining epigenetic therapy with immunotherapy.

[4] FASD: AN EPIGENETIC NEURODEVELOPMENTAL DISORDER MODELLED IN MICE

Bonnie Alberry, Shiva M. Singh
University of Western Ontario

Most neurodevelopmental disorders involve genes, environment and poorly understood epigenetic interactions that are multifaceted, dynamic and complex. Clarifying these interactions is critical but difficult, requiring a suitable model. We propose Fetal Alcohol Spectrum Disorder (FASD), a common neurodevelopmental disorder resulting from prenatal alcohol exposure modeled in C57BL/6J, is highly suited for this. Here we demonstrate that following continuous mild prenatal alcohol exposure in mice during neurodevelopment, mice have behavior phenotypes seen in FASD. Also, they have many life-long hippocampal molecular changes including genome-wide gene and microRNA expression (RNA-Seq), and DNA methylation (meDIP-Seq). The relationship between these features is not understood and may account for initiation as well as persistence of behavioral abnormalities in FASD. Interestingly, changes in DNA methylation in response to prenatal alcohol exposure in mice have also been demonstrated in buccal swabs from children with FASD. More importantly, postnatal manipulations or treatment paradigms potentially applicable to children can be applied and tested in young mice during development. In fact, it is possible to improve or worsen behavioral outcomes by favorable and unfavorable postnatal environments in mice subject to prenatal ethanol exposure, in this case early life stress via maternal separation. Additionally, behavioral outcomes are reflected in newly established molecular features in response to postnatal environment. Critical genes and pathways identified may provide a foundation for the development of FASD diagnosis using DNA methylation and favorable interventions for children born with FASD. Finally, pathways and ontologies associated with these features implicate a role for epigenetic regulation of gene expression common in neurodevelopmental disorders.

[5] FUNCTIONAL CHARACTERIZATION OF TRANSCRIPTIONAL REGULATORS BY SITE-SPECIFIC PROTEIN RECRUITMENT

Nader Alerasool[1] and Mikko Taipale[1]
[1]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto

Understanding the role transcription factors play in controlling gene expression is pivotal to better understanding all biological processes and many human diseases. These proteins often bind and function in combination with other factors. They can work in concert to generate a range of transcriptional output through affecting chromatin accessibility by patterns of histone modifications, recruiting preinitiation complexes or regulating transcriptional elongation. As such, it has been difficult to decipher their individual regulatory roles in different contexts.
My project involves developing a bottoms-up approach for systematic tethering of respective factors to promoters and measuring their regulatory function directly. I utilize a nuclease-deficient Cas9 (dCas9), retaining its ability to bind genomic DNA guided by a sgRNA. This approach allows evaluation of regulators at different endogenous sites in native context and overcomes limitations of plasmid-based readouts. To circumvent costly and time-consuming arrayed screens, I incorporate a pooled human library of ~15,000 human ORFs that enables quick unbiased profiling. This project provides a platform for systematic analysis of potential transcriptional regulators in a

context-dependent manner. Furthermore, the concepts described here could be applied for functional characterization of factors involved in other regulatory processes such as RNA stability, DNA repair or splicing.

[6] THE LANDSCAPE OF DRIVER MUTATIONS IN CUTANEOUS MELANOMA

Rached Alkallas[2,3,4,*], Mathieu Lajoie[2,*], Hamed S. Najafabadi[3,4], Ian R. Watson[1,2]
*[1]Department of Biochemistry, McGill University, Montreal, QC, Canada H3A 1A3. [2]Goodman Cancer Research Centre, McGill University, Montreal, QC, Canada H3A 1A3. [3]Department of Human Genetics, McGill University, Montreal, QC, Canada H3A 0C7. [4]McGill University and Genome Quebec Innovation Centre, McGill University, Montreal, QC, Canada H3A 0G1, * Equal contribution*

The sensitive and specific detection of positive selection on somatic mutations in sun-exposed melanomas is challenged by an exceptionally high rate of passenger mutations induced by ultraviolet radiation. To achieve a greater sensitivity, we pooled somatic variant calls from five whole genome and whole exome sequencing studies, jointly analyzing over 1000 melanoma samples. We identified 32 genes that exhibited statistically significant (FDR < 0.01) evidence of positive selection, 19 of which were not identified as such by any one of the five studies. Positive selection on mutations in 10 of the 32 genes was supported by co-occurring increases in gene copy number, or loss-of-heterozygosity. The 32 genes were collectively enriched for those involved in the MAPK signaling pathway, cell division, immune evasion, and subunits of the SWI/SNF complexes and MLL complexes. Moreover, mutations in one X-linked gene were exclusive to male patients. To further understand the consequences mutations in the 32 genes, we studied their interplay with the melanoma transcriptome. Using non-negative matrix factorization, we untangled melanoma cell intrinsic and non-melanoma cell gene expression, identifying three intrinsic transcriptional signatures: one was characterized by overexpression of genes involved in oxidative phosphorylation, another by upregulation genes targeted by SMARCA2, a subunit of the SWI/SNF chromatin remodeling complexes, and an invasive signature associated with low expression of the MITF transcription factor. The relationships between these transcriptional signatures and mutations in a subset of the 32 genes demonstrated selective constraints on activating mutations in the KIT protooncogene and highlighted the importance of protein kinase A (PKA) signalling in melanoma. In summary, we present the largest mutation significance analysis in melanoma to clarify the landscape of genetic drivers of this disease.

[7] DISSECTING FEATURES OF EPIGENETIC VARIANTS UNDERLYING CARDIOMETABOLIC RISK USING FULL-RESOLUTION EPIGENOME PROFILING IN REGULATORY ELEMENTS

Fiona Allum[1,2], Åsa K Hedman[3], Xiaojian Shao[1,2], Warren A Cheung[1,2,#a], Jinchu Vijay[1,2], Frédéric Guénard[4], Tony Kwan[1,2], Marie-Michelle Simon[1,2], Elodie Boulier[1,2], Bing Ge[1,2], Valérie Siroux[5], Emmanuelle Bouzigon[6,7], Florence Demenais[6,7], Isabelle Pin[8], The Multiple Tissue Human Expression Resource Consortium, Lars Rönnblom[9], Mark Lathrop[1,2], Mark I McCarthy[10,11,12], Panos Deloukas[13], André Tchernof[4], Tomi Pastinen[1,2,#a], Marie-Claude Vohl[4], Elin Grundberg[1,2,#a*]
[1]McGill University, Canada; [2]McGill University and Genome Quebec Innovation Centre, Canada; [3]Karolinska Institute, Sweden; [4]Université Laval, Canada; [5]Université de Grenoble Alpes, France; [6]Institut National de la Santé et de la Recherche Médicale (INSERM), France; [7]Université Paris Diderot, France; [8]Centre Hospitalier Universitaire (CHU) Grenoble Alpes, France; [9]Uppsala University, Sweden; [10]Wellcome Trust Centre for Human Genetics, United Kingdom; [11]University of Oxford, United Kingdom; [12]Oxford National Institute for Health Research Biomedical Research Centre, UK; [13]Queen Mary University of London, UK; [#a]Children's Mercy Hospitals and Clinics, USA.

Background/Methods: Sparse profiling of CpG methylation in blood by microarrays have identified epigenetic links to common diseases. We apply methylC-capture sequencing (MCC-Seq) in a clinical population of ~400 adipose and blood samples, providing high-resolution methylation profiling (>1.3M CpGs) at regulatory elements.
Results: We link methylation to cardiometabolic risk through associations to circulating lipid levels and identify lipid-associated CpGs with novel localization patterns. We confirm MCC-Seq's fine-mapping potential by contrasting with parallel assessments by microarray in ~600 independent adipose samples from the general population and validate lipid-associated regulatory regions. We then show distinct features of tissue-specific versus tissue-independent regulatory regions. By integrating functional datasets, we identified adipose regulatory regions harboring lipid-CpGs that show putative regulation by GWAS SNPs for the same lipid traits.
Conclusions: Overall, the comprehensive sequencing of regulatory element methylomes reveals a rich landscape of functional variants linked genetically as well as epigenetically to lipid traits.

[8] LCM-SEQ: SINGLE CELL-TYPE TRANSCRIPTOMIC PROFILING IN POST-MORTEM BRAIN OF ABUSED SUICIDES

Daniel Almeida[1,2], Gang Chen[1], Jean-Francois Theroux[1], Zahia Aouabed[1], Maria-Antonietta Davoli[1], Naguib Mechawar[1,3] & Gustavo Turecki[1,3]
[1]McGill Group for Suicide Studies, Douglas Hospital Research Center, Verdun, QC, Canada, [2]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada, [3]Department of Psychiatry, McGill University, Montreal, QC, Canada.

Background: The transcriptome of a cell constitutes an essential piece of cellular identity and accounts for the multifaceted complexity and heterogeneity of cell types within the mammalian brain. Each discrete cellular population is differentially influenced by extrinsic signals from their local environments and neighboring cells. Thus, while some studies have investigated transcriptomic alterations underlying the neurobiology of childhood maltreatment and suicide, the use of bulk-tissue homogenates have masked their ability to determine cell-type specific molecular dysfunctions. Here we employ a cell type specific investigation of transcriptomic alterations, in prefrontal layer V pyramidal neurons, associated specifically with a history of childhood abuse (CA).
Methods: Laser captured microdissection (LCM) was used to isolate prefrontal (BA 10) layer V pyramidal neurons from post-mortem human brain. Subject groups included individuals who

died by suicide with and without a history of severe CA and non-psychiatric controls. RNA sequencing libraries were constructed using the SMARTseq v4 kit, to generate full length cDNA libraries, followed by Illumina's Nextera XT DNA library preparation kit. The abused suicide and control groups were used for sequencing. Fifty libraries were sequenced on the HiSeq 4000 using PE 100bp sequencing at a depth of ~40M reads per subject.
Results: We achieved a mapping efficiency of ~80% and captured a wide distribution of transcripts. Differential gene expression analysis revealed significant (FDR <0.10) dysregulation of a variety of protein coding transcripts in abused suicides compared to controls. Gene Set Enrichment Analysis (GSEA) showed significant enrichment of genes with high CpG density promoters in human brain. Weighted Gene Co-Expression Network Analysis (WGCNA) identified two modules negatively associated with CA. Gene ontology (GO) analysis on these modules revealed that they include genes involved in neuronal structure and signaling.
Conclusions: By employing LCM followed by RNA sequencing we were able to uncover cell type specific changes associated with a history of CA. Furthermore, these results open up an interesting avenue to explore whether cell-type specific epigenetic regulation, through DNA methylation, might explain our expression findings.

[9] MIS16 SWITCHES FUNCTION FROM A HISTONE H4 CHAPERONE TO A CENP-A^{CNP1}-SPECIFIC ASSEMBLY FACTOR THROUGH EIC1 INTERACTION.

Sojin An,[1] Philipp Koldewey,[2] Jennifer Chik,[1,3] Lakxmi Subramanian,[4] and Uhn-Soo Cho[1,3,*]
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The Mis18 complex, composed of Mis16, Eic1, and Mis18 in fission yeast, selectively deposits the centromere-specific histone H3 variant, CENP-A^{Cnp1}, at centromeres. How the intact Mis18 holo-complex oligomerizes and how Mis16, a well-known ubiquitous histone H4 chaperone, plays a centromere-specific role in the Mis18 holo-complex, remain unclear. Here, we report the stoichiometry of the intact Mis18 holo-complex as $(Mis16)_2$:$(Eic1)_2$:$(Mis18)_4$ using analytical ultracentrifugation. We further determine the crystal structure of Schizosaccharomyces pombe Mis16 in complex with the C-terminal portion of Eic1 (Eic1-CT). Notably, Mis16 accommodates Eic1-CT through the binding pocket normally occupied by histone H4, indicating that Eic1 and H4 compete for the same binding site, providing a mechanism for Mis16 to switch its binding partner from histone H4 to Eic1. Thus, our analyses not only determine the stoichiometry of the intact Mis18 holo-complex but also uncover the molecular mechanism by which Mis16 plays a centromere-specific role through Eic1 association.

[10] IDENTIFICATION OF ELEMENTS WHICH CONTROL VARIABLE ESCAPE FROM X-CHROMOSOME INACTIVATION

Bradley P Balaton, Carolyn J Brown
Department of Medical Genetics, University of British Columbia

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 samples. Variably escaping genes provide a unique opportunity to compare genes which are subject to or escaping from XCI, in the same genomic context. Analyzing variable escape genes presents the possibility to identify genetic or epigenetic marks which control escape from XCI. Aggregation of XCI status calls from multiple studies provided a comprehensive list of variably escaping genes for further study. In a panel of 17 female cell lines with clonal inactivation patterns, I analyzed the expression of variably escaping genes and made XCI status calls for each gene per sample. Comparisons of the gene's XCI status with promoter CpG methylation levels for these revealed surprisingly little correlation within a sample. To extend these studies to primary tissues I have mined published methylation datasets, finding that methylation levels at variably escaping genes tended to be at an intermediate level between that seen for genes subject to or escaping from XCI. Using whole genome-seq, RNA-seq and whole genome bisulfite-seq data from the Center for Epigenome Mapping Technologies (CEMT), I have made XCI status calls using expression data and, in the majority of tissues, these XCI status calls correlated well with methylation, indicating that prolonged culture may be the cause of the poor correlation found earlier. I will be integrating additional publicly available datasets to have more power for my statistical tests. I have extended analysis of CEMT samples to genetic and chromatin marks for further correlation with a gene's XCI status. H3K9me3 correlates with XCI status while H3K27me3 is inconsistent across tissues. In embryonic stem cells, variable escape genes tend to lack chromatin marks while genes with a consistent XCI status are found enriched at active or weak promoters. Candidate controlling elements will be assessed using a CRISPR-mediated approach to switch from the escape allele to the inactive allele and see if this will change the gene's XCI status.

[11] CHROMATIN MODIFICATIONS MAY CONTROL THE PRECISION OF REMEMBERED EVENTS

Kasia M. Bieszczad, Sooraz Bylipudi, Sean Tsaur, Elena K. Rotondo, Andrea Shang
Department of Psychology Behavioral & Systems Neuroscience, Rutgers University - New Brunswick, Piscataway, New Jersey USA.

A key question in neuroscience is how the brain encodes precise information from experience. Precision in memory is essential, for example, for language learning and comprehension, which depends on highly precise internal representations and associations of sounds-to-meanings. We have shown that the auditory cortex is a substrate of memories that dictate sound-

cued behavior. Experience-dependent plasticity is known to remodel the cortex to enhance the representation of significant sounds. Interestingly, we have found in a series of studies that epigenetic mechanisms may control sensory cortical remodeling in ways that dictate the precision of memory. In particular, histone acetylation may permit subsequent gene expression events to transform the auditory cortex during consolidation to perpetuate the effects of experience into long-term memory. We show by using post-training treatment of a pharmacological inhibitor of histone deacetylase 3 (RGFP966, Abcam Inc.) in animals learning to associate sounds with meaning that this transformation alters the content of memory. The way that HDAC3-inhibition alters remembered events is by increasing the precision of learned acoustic features. Here, we will discuss from two different animal models, two tests of an HDAC3-mediated transformation of learning-induced plasticity in the auditory system that results in highly specific memory that (a) enables auditory discrimination learning in rats (Bieszczad et al., 2015 J Neurosci; Shang et al., 2018 Behav Brain Res) and (b) enables conspecific vocalization (voice) discrimination in birds (Phan, Gergues et al., Front Sys Neurosci). The consolidation of sensory content (precision, accuracy, amount of detail) into memory is an emerging theme that emphasizes how sensory systems of various modalities and across different species are part and parcel to the acquisition and maintenance of memory that later guide behavior. Of note is that epigenetic molecular mechanisms play a role in the induction of sensory system plasticity, enabling the formation of highly specific content in memory and its associative strength. We have formally proposed a role for epigenetic mechanisms in the sensory accuracy of memory formation, called the informational capture hypothesis (Phan & Bieszczad 2016 Neural Plast). That epigenetic mechanisms could control the entry of sensory features into memory is relevant for a wide variety of psychiatric disorders involving cued-behaviors including sensory processing disorders, autism, drug addiction, and post-traumatic stress.

[12] LTR RETROTRANSPOSONS TRANSCRIBED IN OOCYTES DRIVE SPECIES-SPECIFIC AND HERITABLE CHANGES IN DNA METHYLATION

Julie Brind'Amour[1], Hisato Kobayashi[2*], Julien Richard Albert[1], Kenjiro Shirane[1], Akihiko Sakashita[3], Asuka Kamio[2], Aaron Bogutz[1], Tasuku Koike[3], Mohammad M. Karimi[1], Louis Lefebvre[1], Tomohiro Kono[3] and Matthew C. Lorincz[1*]
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De novo DNA methylation (DNAme) during mouse oogenesis occurs within transcribed regions enriched for H3K36me3. As many oocyte transcripts originate in Long Terminal Repeats (LTRs), which are heterogeneous even between closely related mammals, we examined whether species-specific LTR-initiated transcription units (LITs) shape the oocyte methylome. Here, we identify thousands of syntenic regions in mouse, rat and human that show divergent DNAme associated with private LITs, many of which initiate in lineage-specific LTR-retrotransposons. Furthermore, CpG island (CGI) promoters methylated in mouse and/or rat, but not human oocytes, are embedded within rodent-specific LITs and vice versa. Notably, at a subset of such CGI promoters, DNAme persists on the maternal genome in fertilized and parthenogenetic mouse blastocysts or in human placenta, indicative of species-specific epigenetic inheritance. Polymorphic LITs are also responsible for disparate DNAme at promoter CGIs in distantly related mouse strains, revealing that LITs also promote intra-species divergence in CGI DNAme.

[13] RIBOSOME PROFILING AND TRANSLATOME SEQUENCING OF THE ORBITAL FRONTAL CORTEX OF SUICIDE COMPLETERS WITH A HISTORY OF EARLY LIFE ADVERSITY

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Ribosome profiling (riboseq) is a next generation sequencing technique that captures and profiles ribosomal footprints (RPFs): the fragments of the mRNA transcriptome that are contained under ribosomes as translation occurs (also known as the translatome). This allows riboseq to identify actively translated mRNA regions: and therefore, discrepancies between the translatome and transcriptome (or genome) that are sites of epigenetic regulation. This is of great potential benefit to the study of psychiatric conditions where epigenetic changes are implicated in their phenotype, such as suicide completion, particularly when coupled with early life adversity (ELA). Transcriptomic research has found several genes to be differently methylated in ELA/suicides compared to healthy controls and non-ELA/suicides, particularly in brain areas such as the Orbital frontal Cortex (OFC). However, translatomic research verifying altered proteins synthesis levels at these differently methylated sites has thus far been unexplored and could be used further elucidate the epigenetic mechanisms underlying how ELA contributes to suicide. Furthermore, riboseq of non-tumor human brain tissue appears to be unreported in literature due to difficulty with preserving the process in vivo.
I intend to create a novel riboseq procedure for RFP capture in human brain tissue, where sequencing will be completed on samples from the OFC with ELA/suicide, non-ELA/suicide, and healthy control cohorts. Trials optimizing nuclease cocktails for RPF capture, amplification cycles, RPF fragment purification, and cDNA conversion have been involved in the creation of this riboseq procedure.
Early fragment size analysis suggests that certain nuclease cocktails are successful at capturing the characteristic ~30 bp RPF fragments compared to total RNA controls. Additionally, the triplicate periodicity profile characteristic of RPF capture has been observed in trials post MiSeq sequencing. These results are promising and suggest the success of RPF capture from human brain homogenate. With a feasible procedure established, analysis of differentially translated genes (DTGs) and differentially expressed genes (DEGs) between cohorts remains to be explored. Based on previous epigenetic data, I hypothesize that transcriptomic (DEG) and translatomic (DTG) differences will be found between ELA/suicide brains compared to both non-ELA/suicides and healthy controls.

[14] A COMBINED GENOMIC AND EPIGENOMIC ANALYSIS IDENTIFIES CLINICALLY SIGNIFICANT SUBGROUPS OF WILMS TUMORS

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Introduction: Wilms tumors (WT) are the most common childhood renal. Although cure rates are high, the burden of treatment-related late effects is significant. It is evident from clinical experience that there is a high degree of heterogeneity in the natural history of WT. However, currently known molecular features inadequately explain this heterogeneity. Substratification of WT by genome-wide analysis of DNA methylation, exome variants, and RNA expression may identify patients who would benefit from a reduction in therapy.
Methods: WT-kidney pairs (n=29) and unpaired tumours (n=12) were collected at Sick Kids, Toronto at the time of nephrectomy. An additional 40 WT-kidney pairs were obtained from the Childrens' Oncology Group. DNA methylation arrays were used along with whole exome sequencing and poly-A RNA sequencing.
Results: Unsupervised clustering of DNA methylation and RNA expression data revealed three subgroups of WT: One subgroup ("DIFF") has a DNA methylation profile similar to mature kidney, a second subgroup ("PRO") has genome-wide dysregulation of DNA methylation, and a third subgroup ("INT") has an intermediate profile and most closely represents pre-neoplastic nephrogenic rests. Subgroups DIFF and INT share a pattern of RNA expression while PRO has a unique expression pattern. In PRO, there is overexpression of genes associated with proliferation and genes involved in early renal development including HOX genes. The expression of early developmental genes – as opposed to those associated with proliferation – is correlated with alterations in DNA methylation.
Exome sequencing of the tumors showed that recurrent variants in microRNA processing genes occur almost exclusively in PRO. As well, the overall rate of missense mutations and segmental chromosomal aberrations was higher in PRO suggesting a higher degree of genome instability. Recurrent pathogenic variants in the histone-modifier SETD2 gene were identified in WT for the first time.
WT subgroups are clinically important as no cases of relapsed disease nor high-risk histology occurred in Subgroups DIFF or INT. Conversely, bilateral WT were enriched in DIFF.
Conclusions: A multi-omic analysis separates WT into three subgroups suggesting a new model of tumorigenesis in which early tumors reach an inflection point where they can either undergo differentiation or increase their proliferative capacity. The favorable outcomes in Subgroups DIFF and INT imply that some children with WT may be candidates for a reduction in therapy thereby reducing therapy-related chronic disease. Future work will identify the drivers of increased proliferation in PRO as candidates for targeted therapy.

[15] IHEC DATA PORTAL 2018 UPDATE: COMMUNITY HUBS AND INTEGRATION WITH GALAXY

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The IHEC Data Portal (epigenomesportal.ca/ihec) is the integrative online resource to navigate through datasets produced by the International Human Epigenome Consortium. With over 10,000 human datasets and an average of 250 unique sessions weekly, it is the central access point to visualize and obtain IHEC datasets. In order to increase the quality and accessibility of epigenomic data from IHEC and the community as a whole, we have added many features over the last year, to be included in the October 2018 release of the Portal.
First, we are now offering methods to integrate datasets from the community directly into the IHEC Data Portal. By filling an IHEC Data Hub document (e.g. with the Metadator, epigenomesportal.ca/metadator), any lab can publish their epigenomic datasets and make them available in the Portal for download, visualization and analysis, for instance to assess comparability with existing IHEC data. Next, a tightly coupled instance of the Galaxy Framework is also available. By selecting datasets from IHEC and community hubs, users can launch anonymous Galaxy sessions, and import their own datasets in for further analysis along with IHEC data. New services have also been added, such as a reporting tool on datasets and tracks usage by the public. Lastly, many new datasets will be added in the next release, mostly for the hg38 reference assembly, and existing reference epigenomes will include the metadata improvements efforts of the epiMAP re-analysis project.
The IHEC Data Portal is hosted by GenAP (genap.ca), and 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.

[16] MISSENSE MUTATIONS DISRUPTING THE HELICASE DOMAIN OF CHROMATIN REMODELLER CHD3 CAUSE A NOVEL NEURODEVELOPMENTAL SYNDROME WITH INTELLECTUAL DISABILITY, MACROCEPHALY AND IMPAIRED SPEECH AND LANGUAGE.

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Chromatin remodelling is of crucial importance during neurodevelopment. CHD3, a member of the CHD subfamily of chromatin remodelling ATPases and subunit of the NuRD complex, has recently been shown to be specifically involved in late neural radial migration and layer specification in the developing cortex. Unlike other members of the CHD subfamily, pathogenic alterations in the CHD3 gene have not yet been implicated in a human neurodevelopmental phenotype. Upon

whole genome sequencing of a cohort of children with rare speech disorders, we discovered an individual with a de novo missense variant in CHD3. Through a genotype-driven approach to identify more variants disrupting the gene, we collected 35 individuals with de novo CHD3 mutations and overlapping phenotypes. The majority of the mutations clustered within the characteristic ATPase/helicase domain of the encoded protein. We analyzed the functional impact of several of the identified mutations and demonstrated that they affected ATPase activity and nucleosome remodeling. We implicate de novo mutations of CHD3 in a syndrome that is characterized by intellectual disability, macrocephaly and impaired speech and language.

[17] MITOCHONDRIAL DNA COPY NUMBER (MTDNA-CN) INFLUENCES NUCLEAR DNA METHYLATION AT CPGS ASSOCIATED WITH NEUROACTIVE LIGAND-RECEPTOR PATHWAY INTERACTIONS

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Mitochondrial DNA copy number (mtDNA-CN) has been associated with a variety of aging-related diseases, including all-cause mortality. The mechanism by which mtDNA-CN influences disease may be through regulation of nuclear gene expression via the modification of nDNA methylation. Supporting this, we report the cross-sectional association of mtDNA-CN and nDNA methylation as well as experimental evidence that modulating mtDNA-CN leads to changes in nDNA methylation and expression. nDNA methylation and mtDNA-CN were derived from DNA extracted from buffy coat for 1,567 African Americans (AA) and 940 European Americans (EA) from the Atherosclerosis Risk in Communities (ARIC) study. Twenty-three and fifteen independent CpGs reached epigenome-wide significance in ARIC AA and EA cohorts, respectively. Effect estimates for significant CpGs in ARIC AA and EA were strongly correlated (r=0.7). In addition, five significant CpGs validated in an AA cohort (N=239) from the Cardiovascular Health Study (CHS) ($p<0.05$) and 19 of the 23 CpGs showed the expected direction of effect, however this was not the case for the CHS EA cohort (N=294) where we were not able to validate any CpGs. Mendelian randomization using methylation QTLs for mtDNA-CN associated CpGs as the mediator demonstrated that nDNA methylation at these CpGs does not directly drive alterations in mtDNA-CN (ß=0.001, p=0.83) and there was no enrichment of p-values. In contrast, experimental modification of mtDNA-CN through knockout via CRISPR-Cas9 of TFAM, a regulator of mtDNA replication, demonstrated that modulation of mtDNA-CN drives changes in nDNA methylation and gene expression of specific CpGs/transcripts. Strikingly, the 'neuroactive ligand receptor interaction' KEGG pathway was found to be the top overrepresented pathway in the ARIC meta-analysis (p=5.18E-04), as well as TFAM methylation (p=4.41E-04) and expression (p=4.30E-04) studies. These results show that changes in mtDNA-CN influence nDNA methylation at specific loci and lead to changes in gene expression of specific genes, including those acting in the 'neuroactive ligand receptor interaction' pathway and may lead to impacts on human health and disease via altered cell signaling.

[18] INTEGRATED ANALYSIS OF MICRORNA AND MRNA EXPRESSION PROFILES REVEAL MICRORNA REGULATION OF PUBERTY VIA TRANSCRIPTIONAL REGULATORS IN THE PITUITARY GLAND.

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The onset of puberty exhibits variability in timing which not only differs between the sexes but has been associated with adverse later life outcomes including cardiovascular disease, mental illness and cancer. However, the mechanisms that regulate pubertal onset are not well understood. Recent age at menarche (AAM) GWAS studies have implicated genetic variation near pituitary expressed genes. To discover gene regulatory networks active in males and females during postnatal development of the pituitary gland, we used 3'UTR-seq and small RNA-seq to profile the expression of mRNA and miRNA across four ages across the pubertal transition in the pituitary gland of male and female mice. The most significant gene expression changes we observed were prior to puberty (day 12 to 22; ~400 differentially expressed genes). Similarly, most differential miRNA expression changes were observed when comparing day 12 and later ages (n=40). The pituitary transcriptome also showed abundant sexual dimorphic gene expression peri-puberty (day 27) in males (n=86) and females (n=91). 16 of these genes were previously implicated in AAM GWAS. We also identified 5 miRNAs with sex-biased age-specific expression.
We identified predicted targets of differentially expressed miRNAs which were negatively correlated with gene expression across ages (n=637). 23 negatively correlated miRNA-mRNA pairs contained a gene implicated in AAM GWAS. The miRNAs of two miRNA-transcription factors pairs (miR-149-5p/Klf14, miR-214-5p/Six6) had decreasing expression across development. SIX6, in particular has been shown to mediate transcription of

gonadotrope-specific genes during puberty. We also found that miR-211-5p, predicted to target Jarid2, was more highly expressed in males at day 32. JARID2 is cofactor of PRC2 which has been implicated in the epigenetic control of female pubertal onset by histone methylation.
These initial findings suggest miRNAs indirectly modulate gene expression in pituitary gland during puberty and development by targeting transcriptional and epigenetic regulators, however, the regulatory networks downstream of miRNAs remains to be explored.

[19] FOLATE METABOLISM IS LINKED TO DYNAMIC DNA METHYLATION IN HUMAN SPERM

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DNA methylation patterns in sperm are critical for normal offspring health and are highly unique compared to those of somatic tissues. As some gamete-derived DNA methylation escapes reprogramming in early embryos, epigenetic defects in germ cells may also be transmitted to the next generation. Current techniques to assess DNA methylation show bias towards CpG dense regions and do not target areas of variable or dynamic methylation, those predicted to be environmentally-sensitive. To assess variation in human sperm DNA methylation, we performed deep whole genome bisulfite sequencing on an equimolar pool of sperm DNA from 30 men varying in age, fertility status, MTHFR genotype and exposures. Nearly 940,000 sites possessing intermediate methylation levels, termed dynamic sperm CpGs, were identified. These sites, along with 2.2 million commonly assessed genic and intergenic CpGs, were used to customize a novel human sperm methylation capture panel, allowing for targeted and high coverage sequencing. The panel was then used to examine effects of folate metabolism perturbations on sperm DNA methylation patterning. We first assessed the effect of a common polymorphism in methylenetetrahydrofolate reductase (MTHFR) in a cohort of fertile men. As compared to the MTHFR 677CC (n=13) genotype, the 677TT (n=8) genotype (associated with a 50-60% decrease in enzyme activity) resulted in mainly hypermethylation in sperm. Many of the sites of altered methylation were found to be clustered together, creating regions of differential methylation. A different cohort of infertile men given folic acid supplementation for 6 months was also examined. High dose folic acid treatment exacerbated sperm hypomethylation in MTHFR 677TT (n=6) men. Sites of altered methylation were found in genes enriched for gene ontology (GO) pathways related to nervous system development. In both studies, the vast majority (>80%) of altered methylation was found within the dynamic sperm CpGs sites, which would not have been detected with other currently available techniques. Using of our human sperm methylation capture panel, we discovered differential methylation following conditions affecting folate metabolism, the majority of which were found to be in novel dynamic sperm CpG sites. Our customized panel allows for accurate assessment of sperm DNA methylation profiles at single CpGs with an unprecedented coverage, targets putative susceptible sequences in human sperm and improves our ability to examine environmental impacts on DNA methylation in human sperm. (Supported by CIHR).

[20] EXPRESSION OF H3.3 ONCOHISTONES IN DROSOPHILA MELANOGASTER ALTERS DEVELOPMENT AND COMPROMISES TISSUE-SPECIFIC TRANSCRIPTIONAL REGULATION.

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Post-translational modifications of histone proteins play a major role in the regulation of gene expression and disrupting them causes deleterious consequences. For example, histone Lys-to-Met (K-to-M) mutations called oncohistones, have been identified in several cancers. H3.3 lysine 27-to-methionine (H3.3K27M) has been described as a driver mutation in malignant gliomas whereas lysine 36-to-methionine (H3.3K36M) mutation has been characterized in head and neck squamous cell carcinoma and undifferentiated sarcoma. We established Drosophila models for H3.3K27M and H3.3K36M and compared these two mutations in terms of phenotype observed upon overexpression in different tissues. Expressing either H3K27M or H3K36M, but not wild-type H3.3, in many Drosophila tissues is lethal or induces a deleterious phenotype. For instance, overexpression of H3K27M or H3K36M in eye discs induces rough small eyes. H3K27M reduces H3K27me3 but increases the level of H3K36me2, while the reverse is observed upon expression of H3K36M. Expressing interfering RNA targeting Enhancer of zeste (E(z)), the H3K27 methyltransferase, and ash1, the H3K36 methyltransferase, rescues the detrimental effects of H3 mutations through recovery of H3K36me2 and H3K27me3 levels in H3K27M and H3K36M animals respectively. Genes involved in eye development and the piwi-interacting RNA (piRNA) pathways are aberrantly expressed in K27M and K36M mutants. These results demonstrate the value of the Drosophila model in understanding chromatin signaling and uncovering new potential therapeutic targets.

[21] OVERLAPPING DNA METHYLATION SIGNATURES OF BAF (SWI/SNF) COMPLEX VARIANTS CAUSING NICOLAIDES-BARAITSER AND COFFIN-SIRIS SYNDROMES

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The BAF (BRG1/BRM associated factor) nucleosome remodeling complex regulates early development and is especially important in neuronal differentiation. Genetic variants in BAF genes are associated with several neurodevelopmental disorders. Nicolaides-Baraitser syndrome (NCBRS) is caused by heterozygous variants in SMARCA2 which encodes the BAF core catalytic component. Coffin Siris syndrome (CSS) is caused by heterozygous variants in several BAF genes including ARID1B (DNA interacting) and SMARCB1 (complex stabilization). NCBRS and CSS have significant clinical overlap including facial dysmorphism, digital abnormalities, seizures and intellectual disability. Given the importance of the BAF complex in epigenetic regulation, we hypothesized that A) Individuals with pathogenic SMARCA2 variants have a unique DNA methylation (DNAm) signature that molecularly reflects SMARCA2 loss of function and B) Given their molecular and clinical similarity, the signatures of SMARCA2, ARID1B, and SMARCB1 may show some overlap.
DNAm in whole-blood from individuals with variants in SMARCA2 (n=18), ARID1B (n=8), SMARCB1 (n=2), and neurotypical controls (n=24) was assessed using the Infinium MethylationEPIC BeadChip (Illumina). Raw data were processed in the minfi Bioconductor package. A signature of differentially methylated sites for NCBRS was developed using 8 pathogenic SMARCA2 cases versus controls using limma regression with sex and age as covariates.
A signature of 171 CpGs (adj. $p<0.01$; $\Delta\beta>15\%$) distinguished pathogenic NCBRS cases from controls. This signature was then used to classify: variants of unknown significance (VUS) in 3 patients with NCBRS as pathogenic, 5 variants predicted as benign with controls, and a VUS with a milder clinical phenotype as separate from both cases and controls. Four ARID1B$^{LOF/+}$ CSS cases were also separate from cases and controls using the NCBRS signature. We derived a preliminary ARID1B$^{LOF/+}$ signature using these cases comprised of 106 sites (adj. $p<0.01$; $\Delta\beta>10\%$); it classified 4 ARID1B missense variants (no CSS diagnosis) with controls, and the SMARCA2 pathogenic variants as separate from both cases and controls. The SMARCB1 variants classified as pathogenic using both signatures, clustereing with cases in each. DNAm changes were enriched at genes involved in growth, differentiation, and skeletal development in both signatures.
These results demonstrate a high degree overlap in the molecular outcome of these variants which is paralleled in the clinical phenotype. We plan to obtain additional cases for ARID1B, SMARCA2 and other CSS genes with the goal of developing signatures distinguishing variants within each. These DNAm signatures can be used for precision medicine by providing a means of functionally classifying VUS. In addition, the genes affected by these DNAm changes further our understanding of the downstream targets of specific BAF complex components and will provide novel therapeutics that target epigenetic mechanisms.

[22] INTERACTIONS BETWEEN DNA METHYLATION AND HISTONE MODIFICATIONS IN EPIGENETICS-DRIVEN CANCERS

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Oncogenic mutations have been found in histone genes, e.g. histone 3 (H3) p.K27M[1] and p.K36M[2]. However, the mechanisms of how these histone mutations cause cancers remain unclear. DNA methylation is a highly conserved chromatin mark and is critical for the establishment and maintenance of cell fates. Accordingly, the dysregulation of DNA methylation underlies the hallmarks of cancers[3]. In the H3K36M cells, H3K36me2/me3 are re-distributed in a way that H3K36me3 is globally reduced and H3K36me2 shifts from intergenic to genic regions[2]. We, and others in the field, showed that DNA methyltransferases (DNMTs) such as DNMT3A and DNMT3B bound to H3K36me2/me3 via their PWWP domain to deposit DNA methylation in specific regions[4,5]. The reduction of H3K36 methylation in K36M cells leads to DNA hypomethylation especially in intergenic regions, which potentially causes undifferentiated cells forming tumor. However, it is not clear that how H3K27 methylation affect DNA methylation in K27M cells. The polycomb repressive complex 2 (PRC2), which deposits H3K27 methylation, is often recruited to CpG islands, which are generally devoid of DNA methylation. Nonetheless, DNA methylation and H3K27 methylation are not mutually exclusive[6]. PRC2 was shown to be associated with both DNMTs[7] and DNA demethylase Tet1[8]. Therefore, the relationship between DNA and H3K27 methylation is complex and remains to be elucidated. Here we assessed the effect of H3K27me3 on the distribution of DNA methylation by using the primary K27M tumor cell lines and isogenic CRISPR-edited controls. In K27M cells, H3K27me3 is globally reduced and focally distributed in CpG islands of repressed genes. In the CRISPR-edited cells where the K27M mutation is knocked out, the levels and broad domains of H3K27me3 are restored. We profiled DNA methylation in two K27M and two K27M-KO cell lines using whole-genome bisulfite sequencing. Comparing CpG methylation changes between K27M and KO cells, we observed opposite trends in the two K27M cells: one with increased, and the other with decreased CpG methylation after K27M-KO. Nevertheless, the DNA methylation changes in both cell lines mainly occur in intergenic regions. We hypothesize that the effect of H3K27me3 on DNA methylation is context-dependent and probably through H3K36me2. We will further assess the interactions between H3K27 and H3K36 methylation to gain more insights into the mechanisms of cancers driven by histone mutations.

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[23] NOVEL INTERACTIONS BETWEEN H2BUB1 REGULATORS, RTF1 AND THE PAF COMPLEX, CAN BE MODULATED BY DNA BINDING

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Histone modifications mark and maintain transcriptionally active and repressed chromatin states, with certain modifications, such as histone H2B monoubiquitination (H2Bub1), exclusively associated with RNA polymerase II transcription. Impaired regulation of H2Bub1 is associated with gene regulatory defects and different types of cancer, such as breast cancer. The molecular mechanisms that couple H2Bub1 to transcription, and its role in regulating transcription, remain poorly understood. H2Bub1 is catalyzed by the E2 ubiquitin-conjugating enzyme Rad6 and E3 ubiquitin ligase Bre1. Deposition of H2Bub1 during transcription also requires the Polymerase-Associated Factor (PAF) complex and Rtf1, both conserved regulators of transcription elongation and mRNA processing. To elucidate the mechanisms through which the PAF Complex and Rtf1 couple histone modification to the RNA polymerase II elongation complex, I have developed an in vitro system to study the network of protein-protein interactions connecting the PAF complex, Rtf1, and histone-modifying enzyme complexes derived from the model eukaryote fission yeast. Whereas previous studies have shown interactions between these factors involving the N- and C-terminus of Rtf1, I have focused on novel interactions involving the highly conserved Plus 3 domain of Rtf1. Studies indicate that the Plus 3 domain is involved in Rtf1's recruitment to chromatin through a specific interaction with phosphorylated Spt5, a transcription factor that when phosphorylated, allows the release of paused RNA polymerase II into elongation. I have found additional novel interactors with the Plus 3 domain including specific subunits of the PAF complex, as well as with the Rad6/Bre1 complex and the Set1 histone H3 lysine 4 (H3K4) methyltransferase complex. The Plus 3 domain has also been previously described in a nucleic acid binding role, but the function of this interaction is poorly understood. Further analysis of the novel PAF interaction indicates that it is mutually exclusive with this nucleic acid interaction, shedding light on the potential function of this binding. Additionally, we have identified mutations that perturb these interactions in vitro and are in the process of assessing their effects on Rtf1 function in vivo. Our results highlight potentially novel functions and mechanisms of regulation for the Rtf1 Plus 3 domain in linking histone modification and RNA polymerase II transcription.

[24] NOVEL FIRST TIER DNA METHYLATION-BASED DIAGNOSTIC PLATFORM FOR NEURODEVELOPMENTAL DISORDERS

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Diagnostic genetic testing has evolved dramatically in the last decade with the introduction of next generation sequencing (NGS). While significantly improving diagnostic yield, NGS have magnified some of the challenges of DNA sequence-based testing in clinical genomics, most notably the interpretation of variants of unknown significance (VUS), incomplete coverage of certain genomic regions and detection of small deletions/duplications. Our previous work showed that pathogenic mutations in epigenes (genes involved in epigenetic regulation), such as NSD1, CHD7, KMT2D, KDM5C, are associated with epigene-specific genome-wide DNA methylation (DNAm) signatures as well as the utility of these signatures to serve as functional tools for VUS classification. In the current work, we tested the utility of these DNAm signatures for first tier diagnosis of cases with undiagnosed neurodevelopmental syndromes. We compared the profiles of 2000 methylomes (1600 from the Illumina 450k array and 400 from the Illumina EPIC array) generated in our lab from blood samples of cases with known pathogenic mutations, VUS, undiagnosed cases and controls. We tested each sample against the different epigene-specific DNAm signatures associated with the clinical phenotypes of Sotos (NSD1), Weaver (PRC2 complex genes: EZH2, EED, SUZ12), Kabuki (KMT2D) and susceptibility to Autism (CHD8) syndromes. Using principal component analyses as well as unsupervised hierarchical clustering; genome-wide DNAm data for each case were compared to the disease-specific reference (derived from n=5-10 cases with pathogenic mutations) and a control reference (n=15). This approach allowed us to classify two undiagnosed samples with the NSD1-specific DNAm signature. Research-based NGS analyses on these 2 samples identified pathogenic variants in NSD1, thereby validating the first-tier diagnosis of Sotos using the DNAm signature. A review of the phenotype of these two patients demonstrated macrocephaly, developmental delay and other characteristic features of Sotos syndrome. Similarly, we were able to predict mutations in PRC2 complex genes (n=2), KMT2D (n=1) and CHD8 (n=1); these diagnoses were confirmed by NGS and post hoc clinical review of these cases. These data highlight the potential utility of gene-specific DNAm signatures not only in functionally classifying VUS as pathogenic or benign but also in categorizing individuals by syndromic diagnosis, thereby serving as a first-tier diagnostic platform.

[25] ROLE OF NON-CATALYTIC FUNCTIONS OF TET1 IN EMBRYONIC STEM CELL STATE

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Embryonic stem cell (ESC) pluripotency is tightly regulated by epigenetic mechanisms controlling gene expression. Tet1, a member of the Tet family of enzymes that catalyse the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) promoting DNA demethylation, is highly expressed in ESCs. In addition to its catalytic function that influences DNA methylation levels, Tet1 has catalytically independent roles in ESCs. These functions are mediated via its interaction with other epigenetic modifiers, like Hdac1, post-translational modifiers, such as Ogt1

and Parp1, as well as repressor complexes, PRC2 and Sin3a. To establish the biological significance of catalytic and non-catalytic roles of Tet1 in the epigenetic regulation of gene expression in ESCs and during development, we have generated Tet1 catalytically inactive and knockout ESC lines. These lines exhibited striking phenotypic and molecular differences. In contrast to Tet1 catalytically inactive ESCs, Tet1 knockout ESCs had reduced proliferation and enhanced differentiation towards mesendoderm and trophectoderm lineages during embryoid body formation as well as in teratoma formation assay. Comparison of gene expression profile analysis revealed that the majority of Tet1 target genes are regulated by its non-catalytic functions and that a large subset of them are also targets of the chromatin repressive complexes Sin3a and PRC2. Collectively, our findings suggest that the non-enzymatic functions of Tet1, likely facilitated through Tet1 interactome, are responsible for the bulk of Tet1-mediated gene regulation as well as key biological features of ESC. Future work involving the comparative analysis of methylome and hydroxymethylome of these cells will validate non-catalytic Tet1 target genes that are not regulated by DNA methylation. This study will establish the importance of Tet proteins beyond their role in DNA demethylation in stem cells and during development.

[26] TRANSCRIPTIONAL AND EPIGENETIC CHANGES IN THE BRAIN OF OLD MICE WITH DISRUPTED FOLATE METABOLISM

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Low dietary folate and genetic variants in folate metabolism, such as the methylenetetrahydrofolate reductase (MTHFR) 677 C>T polymorphism, are known to impact brain function and increase the risk for neurodegenerative disorders such as Alzheimer's Disease (AD). Folate is required for the synthesis of the universal methyl donor S-adenosylmethionine (SAM), which is used for DNA and histone methylation reactions, thereby contributing to gene expression regulation. Recent studies propose that alteration of the epigenome is an important mechanism underlying the association between folate metabolic disruptions and the increased risk for cognitive decline and AD. In order to better explore these mechanisms, we investigated the effects of a folate-deficient diet or a control diet on the brain of 10-month- old Mthfr$^{+/-}$ mice, a model for the human MTHFR 677 C>T polymorphism, and control mice (Mthfr$^{+/+}$). We assessed changes in cognitive performance and expression of genes involved in amyloid precursor protein (APP) metabolism, synaptic plasticity, histone acetylation and DNA methylation. We also measured levels of expression of methylation homeostasis genes and of one-carbon metabolites. We observed short-term memory impairment, and gene expression changes in Dnmt3a and the synaptic marker Bdnf due to the Mthfr$^{+/-}$ genotype. The folate-deficient diet led to reduced SAM levels, and altered mRNA levels of Dnmt1, Dnmt3b, Hdac3, Hdac5, the APP-processing enzyme Presenilin-1, and the neurotrophic factors Ngf and Bdnf. Notably, dysregulation of the neurotrophic factor Bdnf and of Psen1 appeared to be associated with changes in DNA methylation levels at their gene promoters. These findings shed further insights into the contribution/role of epigenetics on the cognitive decline associated with genetic and dietary folate metabolic disturbances.

[27] TARGETING C-MYC OVEREXPRESSING LEUKEMIA WITH CARDIAC GLYCOSIDE PROSCILLARIDIN THROUGH DOWNREGULATION OF HISTONE ACETYLTRANSFERASES

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Overexpression of MYC transcription factor drives oncogenesis by causing profound gene dysregulation. Targeting MYC remains a major therapeutic goal in cancer chemotherapy. Here, we identified that proscillaridin, a cardiac glycoside approved for heart failure treatment, targets leukemic cells overexpressing MYC at clinically relevant doses. Proscillaridin induced rapid downregulation of MYC protein levels, reduced proliferation in leukemic cell lines and MYC-dependent leukemic stem cell populations. Transcriptomic profiles of leukemic cell lines after treatment were characterized by downregulation of genes involved in cell replication, MYC pathways and upregulation of differentiation pathways, which mirrored drug-induced antiproliferative effects. Gene reprogramming was associated with a significant loss of lysine acetylation on histone H3 at lysine 9, 14, 18 and 27. Furthermore, acetylome profiling uncovered that acetylation loss was extended to non-histone proteins including MYC itself, several MYC target proteins, and a series of histone acetylation regulators. Proscillaridin specificity towards MYC-driven leukemia was associated with protein downregulation of histone acetyltransferases involved in MYC acetylation including KAT2A, KAT3A, KAT3B, KAT5 and KAT6A. Overall, these results strongly support the repurposing of proscillaridin in MYC overexpressing leukemia and also suggest a novel strategy to indirectly target MYC by inhibiting a subset of histone acetyltransferases involved in MYC stability.

[28] EVALUATION OF A TARGETED CUSTOM CAPTURE BISULFITE SEQUENCING APPROACH

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CpG methylation can be modified by environmental exposures, such as smoking and medication. Differential methylation of CpG sites likely contributes to the etiology of many common complex diseases, as such there is increased interest in evaluating CpG methylation and conducting epigenome-wide association studies. CpG methylation can be assessed using a variety of methods including whole genome bisulfite sequencing which can be prohibitively expensive, pyrosequencing of small regions, or the relatively inexpensive, and commonly utilized chip-based approaches such as Illumina's 27K, 450K and Epic arrays. While cost effective, there are significant limitations of the array-based approaches including the limited number CpG sites that they can assessed and the number of CpG sites that demonstrate variable methylation.
An emerging alternative to array-based approaches, targeted capture bisulfite sequencing can offer several advantages including greater number CpGs that can be assessed, greater percentage of CpG sites with variable methylation, joint sequencing of both SNPs and CpG sites facilitates evaluation of both haplotype and SNP effects.
Methods: We recently piloted the Illumina TruSeq Methyl Capture Epic bisulfite sequencing approach using 24 samples selected from asthma studies. TruSeq is a targeted approach that interrogates ~ 3.34 million CpG sites, that have demonstrated variable methylation based on data from international gene expression/epigenetic consortia. Overall, our mean read depths of 48.71, range 28.3 to 131.56, were higher than the expected 30X. We found that ~50% of CpG sites are variable across samples, which as expected, is much higher than ~9% from the 450K, and the 20% estimated to variable genome-wide. We compared the library content (TruSeq) to Illumina's arrays (27K, 450K and Epic). We found that the majority of CpG sites on the arrays (27K (100%), 450K (98%) and Epic (97.5%)) overlapped with the targeted capture library. However, the proportion of CpG sites with variable methylation is considerably higher 50% TruSeq vs. 9% 450K.
Conclusions: A full analysis of the pilot of 24 samples is currently underway but our initial findings suggest that bisulfite targeted capture sequencing offers a cost-effective alternative to whole genome bisulfite sequencing.

[29] NPM AND NPM-MLF1 INTERACT WITH CHROMATIN REMODELING COMPLEXES AND INFLUENCE THEIR RECRUITMENT TO SPECIFIC GENES

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Nucleophosmin (NPM1) is mutated in one third of all acute myeloid leukemia (AML) and is characterized by its aberrant cytoplasmic localization (NPMc+). NPM can also undergo chromosomal translocations, including the NPM-MLF1, which is detected in myelodysplastic syndrome (MDS) and in ~1% of AML. NPM is reported to influence different mechanisms including gene regulation. The aim of the study is to define how NPM regulates gene expression and determine the effect of NPM-MLF1 on genes regulated by NPM.
NPM and NPM-MLF1 protein interactome were purified in K562 cells by tandem immunoaffinity purification. Partners were identified by MS/MS and confirmed by immunoprecipitation. NPM and NPM-MLF1 interact with the chromatin remodeling complexes NuRD, P/BAF and of the ISWI family. Using Ampli-sequencing assay, we identified genes dysregulated by the KD NPM and abnormally expressed in NPMc+ AML cells. ChIP assay, provided us with the evidence that NPM and NPM-MLF1 can be recruited to transcriptional start site (TSS) of specific genes and can modulate the concomitant recruitment of CHD4/NuRD. Pol II recruitment and histone modification profiles suggest that NPM and NPM-MLF1 can participate to gene activation and repression.
These results provide the first demonstration that NPM and the oncoprotein NPM-MLF1 are directly involved in gene regulation; and (ii) can interact and recruit chromatin remodeling complexes to chromatin. Our results provide new bases to understand and further investigate AML characterized by the presence of the NPM-related oncoproteins.

[30] CHARACTERIZATION OF ATXR5/6 PIP MOTIFS IN PCNA AND NUCLEOSOME BINDING

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Histone lysine methyltransferases (HKMTases) ATXR5/6 (Arabidopsis trithorax-related protein 5/6) play important roles in genomic stability and DNA replication. Previous studies show that the catalytic Su(var), E(z), Trx (SET) domain, the PHD domain, and the proliferating cell nuclear antigen (PCNA)-interacting peptide (PIP) motif contribute to the biological roles of ATXR5/6. Our previous findings established that ATXR5/6 SET domain preferentially methylates histone H3.1 on lysine 27 (H3K27) when incorporated in a nucleosome core particle (NCP) and that its PHD domain is important for HKMTase activity; however, the role of its PIP motif in H3.1 methylation remains to be established. In this study, we show that two molecules of ATXR5/6 bind to a PCNA trimer and the crystal structure of PCNA bound to PIP peptide demonstrates that a di-phenylalanine motif may be important for high affinity binding of ATXR5/6 to PCNA. Surprisingly, increasing concentration of PCNA blocks ATXR5/6 HKMTase activity on the NCP suggesting that the NCP and PCNA have overlapping binding sites on ATXR5. Accordingly, mutations introduced in the PIP motif correspond to decreased H3 methylation by ATXR5/6. Using analytical techniques we also show that 2 molecules of ATXR5/6 bind the NCP and provide preliminary and low resolution CryoEM images of the ATXR5/6:NCP complex. Our studies collectively show that the PIP motif plays an important role in the activity of ATXR5/6 and

suggest that PIP motif may facilitate H3.1 methylation by increasing the affinity of ATXR5/6 with the nucleosome.

[31] DIET-INDUCED OBESITY IMPACTS CHROMATIN REMODELLING IN MYC-DRIVEN PROSTATE CANCER

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Background: Epidemiological studies have reported that systemic metabolic alterations associated with increased intake of saturated fats result in more advanced and lethal prostate cancer (PCa). Recently, gene expression profiling of human prostate tissue identified chromatin regulation as a potential link between obesity and lethal PCa. However, the mechanism underpinning this association remains elusive.
Hypothesis: We hypothesize that diet-induced obesity (DIO) facilitates the progression of PCa toward a lethal phenotype by altering the epigenetic landscape of PCa tumors and increasing chromatin accessibility to pioneer factors.
Results: Using a MYC-driven PCa mouse model, we found that DIO greatly affects the progression of the disease, as demonstrated by an increased tumor burden and cell proliferation by 36 weeks of age compared to mice fed a control diet. At 12 and 24 weeks of age, clinicopathologic features of MYC transformed tissues remained unaltered by DIO. The lack of DIO-dependent phenotype at 12 weeks of age, combined with the robust and uniform transition to prostatic intraepithelial neoplasia (PIN) triggered by MYC over expression, led us to investigate alterations driven by DIO before the appearance of a DIO-dependent phenotype. Genome-wide mRNA profiling (RNA-seq) of 12-week-old MYC-driven prostate tumors revealed that DIO enriches for gene sets implicated in chromatin function. Using the assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq), we generated a genome-wide map of open chromatin regions and found that tissue transformation was associated with an increase in chromatin accessibility to regions associated with DNA motifs encoding binding sites for pioneer factors, such as the forkhead box A1 (FOXA1), which has a demonstrated role in PCa oncogenesis. Importantly, these features were further enhanced by DIO. Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) revealed that FOXA1 cistrome is indeed extensively reprogramed following MYC-driven prostate transformation, but to a greater extent in animals under the DIO regimen. Additional interrogation of open chromatin regions by ATAC-seq revealed that increased accessibility to pioneer factors binding sites associated to MYC-driven tissue transformation (12 weeks) requires DIO in order to be maintained as the disease progresses (24 and 36 weeks).
Conclusion: Collectively, our results suggest that DIO strengthens the establishment and supports the sustained enhancement of a FOXA1-dependent transcriptional program that promotes the progression of PCa toward a more aggressive and lethal phenotype. Experiments are currently ongoing to validate if DIO induces prostate tumor addiction to a FOXA1-dependent transcriptional program.

[32] DEVELOPMENT OF A SHORT-TERM EPIGENETIC IN VITRO ASSAY FOR THE IDENTIFICATION OF CARCINOGENS.

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Regulatory agencies rely on two-year rodent cancer bioassays to identify chemicals that are carcinogens, but for ethical and financial reasons such bioassays are not as frequently conducted. Considering that epigenomic anomalies are critical events in carcinogenesis, and as there is no other approved method to identify non-genotoxic carcinogens, this creates an urgent need to develop new and complementary cancer bioassays. The overarching goal of this project is to identify early epigenetic markers that could permit the identification of carcinogens that act through either genotoxic or non-genotoxic mechanisms. For the first objective, epigenetic changes will be identified in Syrian hamster fetal cells (SHFC) that have bypassed senescence, as an essential early oncogenic step. Using these epigenetic marks, the second objective is to conduct time series experiments starting with primary SHFC to determine which of these marks become detectable at the earliest time following exposures to carcinogens and are conducive to senescence bypass. These epigenetic changes could provide critical biomarkers of carcinogenesis.
The SHFC was selected as a model because these are primary normal cells (normal karyotype and intact DNA repair system), which upon exposure to carcinogens generate morphologically transformed foci (MTF) within a 7-day in vitro exposure experiment. These MTF can bypass senescence and become tumorigenic in syngeneic animals. To fulfill the first objective, MTF were obtained after exposure to the genotoxic carcinogen benzo[a]pyrene and then grown until the cells bypassed senescence. Two such clones were compared to primary cells for DNA methylation differences using pyrosequencing-based assays including a global genome luminometric assay (LUMA), and other assays targeting DNA repeated sequences (DRS) and tumor suppressor gene promoters (p16, E- and P-cadherin, MBD2). SHF clones and primary cells from females were analysed using reduced representation bisulphite sequencing (RRBS), which indicated global genome hypomethylation and genes with differentially methylated promoters requiring further validation. Global genome DNA hypomethylation by RRBS

supported a persistent 40% drop in DNA methylation in DRS after passage 7 of the benzo[a]pyrene-induced clones.
Overall, our preliminary results suggest that global genome and DRS hypomethylation differentiate MTF-derived clones from primary cells and thus may serve as early indicators of oncogenic potential. Future work with RRBS will be undertaken to determine if these endpoints, and additional RRBS identified epigenetic marks, are predictive biomarkers for carcinogenesis.

[33] IDENTIFYING CRITICAL REGULATORS IN TRIPLE-NEGATIVE BREAST CANCER

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Breast cancer is the second leading cause of cancer death in women worldwide. 15-20% of breast cancer patients are diagnosed with triple-negative breast cancer (TNBC) which is negative for receptors estrogen and progesterone, and for overexpression of HER2 gene. Importantly, TNBC is the aggressive subtype of breast cancer and do not respond to the targeted therapies. Integrative clustering (iC) of primary breast tumors revealed distinct subgroups of TNBC*. However, we know very little about the functional differences across these subgroups, limiting the translation of TNBC subgrouping into new effective therapeutic strategies. To address this limitation, we intend to delineate transcription pathway dependencies based on the cis-regulatory landscape across TNBC subgroups. 19 TNBC cells lines and 17 TNBC patient-derived xenografts (PDXs) were assessed for genome-wide chromatin accessibility and gene expression profiling using ATAC-seq and RNA-seq. We identified 166,628 regions accessible in at least one TNBC cell line. Of those, 32% were defined as TNBC specific; not shared by DNaseI-hypersensitive sites in luminal subtype identified by ENCODE Project Consortium. We assigned the TNBC cell lines to four integrative clusters (iC3, iC4, iC9 and iC10) of breast cancer tumors based on their gene expression profiles. Then, we determined the subtype-specific enrichment of chromatin accessibility across these subgroups. 18.6%, 50.8%, 56.4% and 40.8% of the exclusively accessible chromatin regions were TNBC specific in iC3, iC4, iC9 and iC10, respectively. Cluster specific accessible regions showed distinct set of transcription factor motif enrichments. The most enriched motifs in TNBC specific and exclusively accessible regions included the binding site motifs for CEBP, ETS, SOX and P53 families in iC3, iC4, iC9 and iC10, respectively. In the cluster specific regions shared by luminal subtype, FOX family members were the most enriched motifs in iC3, and GATA members in iC4. In addition, we identified 131,872 regions accessible in the ATAC-seq catalogue of TNBC PDXs. Now we are extending our analysis to characterize genome-wide chromatin accessibility of TNBC PDXs and to integrate the findings with gene essentiality data sets**. Together, this study develops an understanding of transcriptional mechanisms driving the unique identity across TNBC subgroups. This helps identify novel TNBC specific drug targets.

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[34] IDENTIFYING THE ROLE PROTEIN ARGININE METHYL TRANSFERASE 1 IN MUSCLE STEM CELL FUNCTION

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Muscle wasting diseases encompass a broad spectrum of phenotypically distinct diseases. Duchenne muscular dystrophy (DMD) results from a mutation in the gene encoding dystrophin. DMD is a progressive disease where the patient succumbs by the 3rd decade of life. In an effort to rebuild damaged muscle, muscle stem cells (MuSCs) differentiate to produce new muscle. Muscle wasting occurs when the MuSC pool is exhausted and can no longer regenerate muscle. There are several intertwined molecular steps outside of the genetic mutation that ultimately lead to the disease phenotype in DMD. Developing therapies must first involve elucidating which molecular pathways regulate MuSC differentiation and self-renewal. Epigenetics play a crucial role in MuSC homeostasis. Histone modifiers, chromatin structure, and transcription factors are all components of the epigenetic machinery that drives MuSC function. Key components of this epigenetic machinery are protein arginine methyl transferases (PRMTs). Recent evidence suggests that PRMT1 in particular is highly entangled in the myogenic process. For instance, we have shown that PRMT1 effects MuSC fate by regulating the transcriptional activation of the major myogenic transcription factor MyoD. Knocking out PRMT1 in mouse MuSCs prevented differentiation and enhanced proliferation. Based on these findings, we hypothesize that transient PRMT1 inhibition will allow for expansion of the MuSC pool, thus priming the muscle for enhanced regeneration following injury once inhibition is de-repressed. We have shown that transient inhibition of PRMT1 in mouse primary myoblasts can be achieved with MS023, an inhibitor of type I PRMTs. Further, treatment with MS023 results in enhanced proliferation of myoblasts in vitro. Interestingly, this phenotype is recapitulated on individual muscle fibres that have been cultured with MS023. Treated fibres have more associated MuSCs than untreated fibres. To investigate the epigenetic consequences of PRMT1 inhibition and elucidate a mechanism by which proliferation is being affected, ChIP-seq is being performed in the presence and absence of MS023 in C2C12 myoblasts. This will yield an overview of the genome-wide placement of PRMT1 and its associated histone mark H4R3me2a when cells are treated and undergoing enhanced proliferation. To assess MS023 in vivo, MuSCs will be isolated from mice, treated with MS023, and engrafted into recipient mice. Following engraftment, muscle injury will be induced and regeneration will occur for 3 weeks. Cross-sections of the injured muscle will be assessed to observe fibre structure and MuSC number. We expect that the mice with treated MuSCs will have an expanded MuSC pool following regeneration compared to mice with untreated MuSCs. We expect that these data will identify the inhibition of PRMT1 as a promising avenue to explore for the replenishment of the MuSC pool and ultimately the treatment of muscle wasting disease. This work is funded by the CIHR.

[35] EPIGENETIC DISCRIMINATION: EMERGING APPLICATIONS OF EPIGENETICS POINTING TO THE LIMITATIONS OF POLICIES AGAINST GENETIC DISCRIMINATION

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Over more than two decades, various policies have been adopted worldwide to restrict the use of individual genetic information for non-medical reasons by third parties and prevent 'genetic discrimination'. In this paper, we bring attention to the growing interest for individual epigenetic information by insurers and forensic scientists. We question whether such interest could lead to 'epigenetic discrimination' – the differential adverse treatment or abusive profiling of individuals or groups based on their actual or presumed epigenetic characteristics – and argue that we might already be facing the limitations of recently adopted normative approaches against genetic discrimination. First, we highlight some similarities and differences between genetic and epigenetic modifications, and stress potential challenges to regulating epigenetic discrimination. Second, we argue that most existing normative approaches against genetic discrimination fall short in providing oversight into the field of epigenetics. We conclude with a call for discussion on the issue, and the development of comprehensive and forward-looking preventive strategies against epigenetic discrimination. Keywords: epigenetics, DNA methylation, discrimination, insurance, forensic science, ethics, justice, policy

Reference

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[36] GENOME WIDE META-ANALYSIS OF PARENT-OF-ORIGIN EFFECTS OF ASTHMA, ATOPY AND AIRWAY HYPERRESPONSIVENESS IN FOUR COHORTS

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Background: The main genetic effects of the common SNPs identified by Genome-Wide Association Studies (GWAS) do not fully explain the heritability of asthma. Genomic imprinting (parent-of-origin effects) is a potential mechanism that may explain this missing heritability.
Aim: Identify candidate genomic regions for imprinting in asthma, atopy and airway hyperresponsiveness (AHR).
Methods: We used GWAS data from four family-based studies (trios): Canadian Asthma Primary Prevention Study (CAPPS), Study of Asthma Genes and Environment (SAGE), Saguenay-Lac-Saint-Jean Québec Familial Collection (SLSJ), and 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. An odds ratio for parent-of-origin effects is determined by dividing maternal odds ratio by paternal odds ratio. Meta-analysis was conducted using the parent-of-origin effects results of SLSJ, DAG, and the joint analysis of the two birth cohorts CAPPS and SAGE (CAPPS/SAGE), weighted by the number of informative transmissions for each study.
Results: Meta-analysis for asthma, using results of SLSJ (251 trios), DAG (316 trios), and CAPPS/SAGE (141 trios), resulted in 5 independent SNPs with significant parent-of-origin effects with P $\leq 1.49\times10^{-5}$ (suggestive P-value threshold). Meta-analysis for atopy, using results of SLSJ (229 trios), DAG (312 trios) and CAPPS/SAGE (217 trios) resulted in 2 independent SNPs. Meta-analysis for AHR using results of SLSJ (132 trios), DAG (260 trios) and CAPPS/SAGE (219 trios) resulted in 7 independent SNPs. Of the significant results, 11 out of 14 of the SNPs were in or near long non-coding (lnc)RNA genes.
Conclusions and future directions: Our results suggest a possible role for lncRNAs in parent-of-origin effects in asthma and allergic phenotypes. We will conduct further analyses based on multinomial modeling and haplotype estimation (using EMIM tool). EMIM allows for the inclusion of ambiguous trios using haplotype estimation (using SHAPEIT2), which increases sample size and potentially the overall power of the study.

[37] IDENTIFYING THE MOLECULAR MECHANISMS UNDERLYING SOCIALLY-MEDIATED PUBERTAL SUPPRESSION

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Pubertal timing is highly heritable and directly influenced by a myriad of environmental conditions including psychosocial stressors. While the mechanisms that control of pubertal timing are only beginning to be understood, epigenetic mechanisms have been clearly implicated.
The naked mole-rat (NMR) is a unique rodent exhibiting extreme socially-mediated reproductive suppression. NMRs reside in large colonies of adults who remain in a pre-pubertal state due to the presence of a single, dominant breeding pair. Most NMRs will never go through puberty unless they are removed from the suppressive cues of their colony. To discover molecular mechanisms contributing to socially-mediated reproductive

suppression we are comparing gene expression and epigenetic profiles in candidate tissues obtained from males and females within the colony (breeding pairs and subordinates) to sex-matched animals that have been removed from their colony for 4 weeks, which triggers pubertal onset. Using RNA-seq we are profiling six reproductively- and socially-relevant regions of the brain as well as the pituitary and gonads. At present, we have profiled the transcriptome of the dorsal hypothalamus (stress inputs) and ventral hypothalamus (control of pubertal timing). In the ventral hypothalamus we have found transcription factors (EBF3 and TCF7L2) and genes encoding hormones (e.g. AVP, TRH, GNRH1) that seem to be upregulated in female subordinates that were removed from the colony and have initiated puberty. We also observe enrichment of dopaminergic pathways in the ventral hypothalamus of female breeders relative to non-dominant females (subordinates in-colony or removed from colony). Epigenomic profiling comparisons within the hypothalamus are currently underway.

[38] ASSOCIATION BETWEEN GENOME-WIDE DNA METHYLATION PATTERN AND RESPONSE TO TRASTUZUMAB IN HER2-POSITIVE BREAST CANCER PATIENTS

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Introduction. The administration of trastuzumab has led to significant improvement in survival of HER2-positive breast cancer patients in the adjuvant and metastatic settings. Trastuzumab resistance, however, has been increasingly recognized as a major obstacle. Recent evidence suggests that epigenetic mechanisms might be associated with acquired resistance to cancer therapies. Aim of this study was to explore the association between genome-wide DNA methylation pattern in breast cancer tissue and the response to trastuzumab.
Patients and methods. DNA methylation pattern was assessed in breast cancer tissues of trastuzumab-treated HER2-positive breast cancer patients who acquired resistance to treatment (case group, n=6) and compared to that of trastuzumab-treated HER2-positive breast cancer patients who did not develop resistance (control group, n=6) using the Illumina Infinium HumanMethylation450 BeadChip. Cases were matched to controls for several factors. Bioinformatics analyses were performed using the R statistical environment (robust linear regression method) to identify differentially methylated genes (DMGs) (FDR < 0.05) between case and control groups.
Results. Compared to the control group, in the matched case group we identified 879 hypermethylated and 293 hypomethylated genes. The differentially methylated set of genes was enriched in molecular and cellular functions associated with cellular movement (P=8.02E-07) as well as cell death and survival (P=4.24E-09). Pathways associated with ERK/MAPK signaling and regulation of the epithelial-mesenchymal transition were overrepresented (P=2.13E-05, and P=2.43E-03, respectively). Among the DMGs we observed AGPAT1, a gene related to the PI3K-mTOR pathway which has been reported to be implicated with trastuzumab resistance in addition to be differentially expressed between HER2-positive breast cancer patients who responded to trastuzumab and those who acquired resistance.
Conclusions. Although our sample size was small, we observed DMGs associated with the response to trastuzumab. These data need to be confirmed in a larger prospective cohort of trastuzumab-treated HER2-positive breast cancer patients.

[39] DISSECTING THE REGULATORY FUNCTIONS OF PRC1 AND PRC2

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The Polycomb group proteins were initially identified as repressors of developmental genes. In mammals, the Polycomb group proteins are found in two large multi-protein complexes, Polycomb Repressive Complexes (PRCs) 1 and 2. Despite extensive study of these repressive complexes, understanding the mechanisms underlying their recruitment and requirement for gene repression still needs further investigation. Using the developing mouse limb as a model system, we aim to study, in vivo, the contribution of PRC1 and PRC2 to gene repression and the functional relationship between the two complexes genome-wide. Genomic techniques such as ChIP-seq and RNA-seq were carried out along with the limb conditional inactivation of PRC2 to assess the role of PRC1 and PRC2 in transcriptional regulation. These genome-wide analyses revealed an interesting relationship between PRC1 and PRC2 and demonstrated an unexpected role for PRC1 at active genes. Overall, these genome-wide studies shed light on the complex nature of gene repression and provide insight into the regulatory functions of Polycomb group proteins during development.

[40] IMPACT OF USING A PERSONALIZED GENOME ON HISTONE CHIP-SEQ PEAK CALLS

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Next generation sequencing experiments typically involve the alignment of reads to a reference genome, a composite made from genomic sequences of multiple individuals. However, differences from the reference sequence are present in all individuals, because of the genetic diversity observed as single nucleotide substitutions and structural variants, such as insertions and deletions. We hypothesized that using a genomic reference that does not account for variations can lead to incorrectly mapped reads and biased downstream results such as ChIP-seq peak calls. To demonstrate this, we show that accounting for genetic variation using a modified reference

genome (MPG) or a denovo personalized genome (DPG) can alter histone (H3K4me1, H3K27ac) ChIP-seq peak calls by either creating new personal peaks or by the loss of reference peaks. The best MPGs are found to alter roughly 1% of peak calls while DPGs can produce up to 5 times more altered peak calls. At the same time, we show differences beyond the calls in terms of read counts in regions associated with the new, altered and unchanged peaks. Such differences between the reference and personalized alignments are abundant in DPGs but rarely seen in MPGs. We notice that indels, followed by SNVs have the highest chance to change peak calls in MPGs. A counter-balancing factor is peak width, with wider calls being less likely to be altered. Read length also influences the impact of a personalized genome on histone peak tracks. Longer reads quickly reduce the impact in MPGs whereas DPGs maintain a strong impact despite fluctuations due to read length. Overall, the most significant altered peaks originate from DPGs, possibly due to the capture of SVs that are not present in MPGs. This suggests that the quality of personalized genomes may benefit greatly from the incorporation of SVs in addition to SNVs and indels.

[41] CHOLINE CONTENT IN THE GESTATIONAL DIET PROGRAMS HYPOTHALAMIC ENERGY BALANCE PATHWAYS IN MALE WISTAR RAT OFFSPRING

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The effects of imbalanced nutrition during pregnancy on the child's predisposition to develop chronic diseases later in life, have been primarily documented by dietary alterations in the mother's total nutrient intake. Our lab was the first to report that feeding pregnant Wistar rats high multivitamin and/or folic acid (FA) diets predispose male offspring towards obesity and components of the metabolic syndrome. These effects have been attributed to DNA methylation-dependent epigenetic regulation of hypothalamic appetite regulatory systems. FA and other methyl nutrients (e.g. choline), are critical components of the 1-carbon cycle which tightly regulates the transfer of 1-carbon moieties for DNA methylation reactions. Contrary to the excessive intakes of FA reported in women of childbearing age, an opposite trend is observed for choline. Choline has a critical role in the epigenetic regulation of brain development and in contrast to cognitive function, the role of choline in the development of physiological functions regulated by the hypothalamus has not been explored. This is the first study to elucidate the role of gestational choline on the in-utero programming of the hypothalamus and the regulation of energy balance in male Wistar rat offspring.
Pregnant Wistar rats (n=14/group) received an AIN93G diet with recommended choline (RC, control, 1 g/kg diet), low choline (LC, 0.5-fold RC), or high choline (HC, 2.5-fold RC) and then the control diet during lactation. Male offspring were fed the control diet up to 17-weeks then terminated. At birth, protein expression of hypothalamic food intake neurons, plasma glucoregulatory hormones and brain levels of S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), choline and metabolites were assessed in male pups. Post-weaning food intake, body weight-gain and 24-hour energy expenditure were measured.
At birth, brain choline levels reflected the choline content of the gestational diet however, choline metabolites and levels of SAM and SAH were not affected. HC pups had higher hypothalamic protein expression of the anorexigenic pro-opiomelanocortin and the orexigenic neuropeptide-Y neurons than both groups ($p<0.01$) whereas LC offspring had lower leptin receptor ($p<0.05$). Both HC and LC pups had reduced plasma leptin than RC pups at birth ($p<0.01$). During adulthood, HC offspring had higher FI compared to RC (11%, $p<0.01$), and higher body weight-gain than both groups (12%, $p<0.05$). Moreover, LC offspring had lower energy expenditure and locomotor activity than both groups (6%, $p<0.05$).
In conclusion, below and above recommended intakes of choline during gestation differentially alter in utero development of central food intake systems, body weight-gain and energy expenditure in mature rat offspring. Whether these effects are associated with choline-dependent regulation of DNA methylation are currently being explored.

[42] H3K27M AFFECTS GENOME-WIDE H3K27ME2 AND H3K27ME3 DISTRIBUTION AND IS ESSENTIAL FOR GLIOMA TUMORIGENESIS

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High-Grade Gliomas (HGG) are devastating brain tumors and a major cause of cancer-related mortality. Pediatric HGG have distinct molecular alterations compared to adult HGG, notably frequent somatic mutations in histone 3 (H3) genes. The most frequent histone mutation, H3K27M, specifies diffuse midline gliomas. Mutant H3K27M, which can occur in both the canonical (H3.1/2) and non-canonical (H3.3) histone variants, contributes to only a small fraction of the total H3 pool. However, it has been shown to have a dominant effect as it drastically reduces overall levels of the repressive H3K27me3 mark in cells. How H3K27M contributes to tumorigenesis, as well as its requirement for tumor maintenance, remain controversial. In addition, there is no clear consensus regarding the mechanism of H3K27M action leading to global epigenomic changes.
We aim to better understand the molecular and cellular effects of H3K27M mutation using primary pediatric HGG cell lines and isogenic controls, assessing H3K27M effects in vitro and in vivo. We directly manipulated H3K27M by overexpressing it in wild-type cell lines or using CRISPR editing to remove it from mutant cell lines. We used histone mass spectrometry, epigenome and transcriptome analysis to study the molecular consequences, cell assays to profile proliferation, and a mouse orthotopic xenograft model to determine the effect of the mutation on tumorigenicity.
Our data show that the main effect of the H3K27M mutation is to prevent the spread of H3K27me3, and to a lesser extent

H3K27me2, from Polycomb Repressive Complex 2 (PRC2) binding sites to larger silencing domains. Whereas H3K27me3 is normally deposited across broad regions, in H3K27M cells it is restricted to PRC2 nucleation sites at large unmethylated CpG islands. These epigenetic changes are reversible upon removal of the H3K27M mutation. The effect of H3K27M can be partially rescued by the mutant EZH2-Y641N variant that preferentially catalyzes H3K27me2 to -me3. Loss of H3K27me3 has limited transcriptomic consequences, preferentially affecting lowly-expressed genes that regulate neurogenesis. Removal of H3K27M from tumor cells restores H3K27me2/3 spread, impairs cell proliferation, and importantly, completely abrogates their capacity to form tumors in mouse brain xenografts.
In summary, our results clarify previous models in the field, importantly, refuting the hypothesis of PRC2 being attracted and held by H3K27M. We provide thorough epigenomic characterization of H3K27M mutation effects, including description of H3K27me2 changes not studied previously, and demonstrate the necessity of H3K27M mutation for HGG cells to maintain their growth and epigenomic landscape.

[43] ASSESSING EPIGENETIC INTRA-TUMOUR HETEROGENEITY THROUGH DNA METHYLATION ENTROPY

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Purpose: There have been several attempts to quantify DNA methylation heterogeneity in cancer samples to analyze their relationships to patient outcomes. Previously proposed measures, such as proportion of sites with intermediate methylation and normalized entropy, are site-specific measures that ignore surrounding nucleotides, and may not capture the group-like nature of DNA methylation. This work builds on these measures, and details a read-based, information theoretic framework to address drawbacks in other measures of heterogeneity.

Methods: We use reduced-representation bisulfite sequencing (RRBS) data from the ENCODE Consortium to measure DNA methylation in various cell lines and develop a measure of DNA methylation heterogeneity from this data source based on information theory. This measure is compared between biological replicates and different cell lines to test sensitivity and robustness of the measure.

Conclusions: This distributed entropy measure more faithfully captures the population level heterogeneity in DNA methylation patterns than other measures and detect population differences in artificially mixed tissue samples. Continuing this work will help elucidate intra-tumour heterogeneity that arises in the epigenome in cancers.

[44] POST-TRANSLATIONAL MODIFICATIONS OF NEW HISTONE MOLECULES PRIOR TO THEIR DEPOSITION AT REPLICATION FORKS

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Introduction: In proliferating cells, chromatin structures that carry epigenetic marks must be faithfully transmitted from parental cells to daughter cells. An important obstacle to high-fidelity transmission of epigenetic marks is that chromatin is transiently disrupted during DNA replication. The fidelity of epigenetic mark transmission therefore depends upon restoration of chromatin structures in the wake of DNA replication forks. The first step in chromatin structure restoration is the rapid deposition of new H3-H4 molecules onto newly replicated DNA. This is followed by a poorly understood, but crucial step that requires "Xerox" enzymes to copy epigenetic marks from pre-existing histones onto the new histone molecules deposited at the same locus. We are studying this key event in epigenetic mark transmission in Schizosaccharomyces pombe. In this organism, the epigenetic nature of H3K9 methylation has been rigorously proven [Science 348: 132]. Chromatin Assembly Factor 1 (CAF1) is a protein conserved from yeast to humans that deposits new H3-H4 molecules onto nascent DNA.
Experimental design, controls and results: In order to define the spectrum of modifications present in new H3-H4 molecules prior to their deposition onto nascent DNA, we designed a simple purification strategy to isolate CAF1-H3-H4 complexes and determine the abundance of modifications at specific residues using protein chemistry and mass spectrometry (MS). Relative to a mock purification from a strain where CAF1 was not epitope tagged, our MS results showed that two known binding partners of CAF1, namely PCNA and H3-H4, were enriched 100- to 150-fold when co-purified with epitope-tagged CAF1.
Di-acetylation of K5 and K12 was the most abundant pattern of acetylation in H4 molecules co-purified with CAF1. This is an important observation because di-acetylation of H4K5 and K12 is a hallmark of new H4 molecules in many species. This result suggests that the majority of H4 molecules co-purified with CAF-1 are newly synthesized, rather than chromatin-derived histones.
H3 molecules co-purified with CAF1 were acetylated at several lysine residues (H3K9, K14, K23, and K56) but, importantly, H3K9 methylation was not detected in CAF1-associated histones. This suggests that H3K9 methylation is absent from new H3 molecules prior to their deposition at replication forks. In contrast, H3K9 methylation was detected in total histones isolated from the same cell culture as the histones co-purified with CAF1.
Conclusion: Those results are consistent with the hypothesis that H3K9 methylation needs to be faithfully copied from pre-existing histones onto new H3 molecules for high-fidelity transmission of epigenetic marks in proliferating cells. I hope to identify the "Xerox" enzyme(s) that performs this crucial step in epigenetic inheritance and decipher its mode of action.

[45] INTERPLAY OF HISTONE AND DNA MODIFICATIONS IN CANCERS WITH IMPAIRED H3K36 METHYLATION

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Enzymes catalyzing histone post-transcriptional modifications (PTMs), including the Nuclear receptor binding SET-Domain 1 (NSD1) and the multiprotein enzyme complex PRC2, are

indispensable for multicellular development and homeostasis. Perturbations of their function have been implicated in human disorders and cancer. For instance, inhibition of PRC2 activity caused by H3K27M is involved in two pediatric brain cancers, diffuse intrinsic pontine gliomas (DIPGs) and supratentorial glioblastoma multiforme (GBMs). Also, mutations in the NSD1 gene are responsible for Sotos Syndrome, a disorder characterized by an overgrowth phenotype in childhood in which a reduction in intergenic DNA methylation have been observed. NSD1 alterations have also been identified in human papillomavirus (HPV)-negative head and neck squamous cell carcinomas (HNSCCs). Our group has recently shown convergent downstream effects of NSD1 and H3K36M mutations, resulting in the reduction of H3K36me2, DNA methylation, and overexpression of a subset of LTR elements. Our objective here is to better understand the relationship between NSD1, PRC2 and their respective effect on H3K36 and H3K27 marks together with global DNA methylation. Using two HNSCC cell lines carrying NSD1 truncating mutations (SKN-3 and SCC-4) and three cell lines bearing wild-type NSD1 (FaDu, Detroit562 and Cal27) we demonstrate that a drastic and global decrease in DNA methylation at intergenic regions is directly associated with the lack of H3K36me2 mark. Furthermore, we observe that the decrease of H3K36me2 mark in intergenic regions is correlated with increased H3K27me3 in those same regions. In order to understand better this antagonistic effect and validate the relationship between NSD1 and H3K36me2 mark, we are currently in the process of mutating NSD1 gene in two of the wild-type NSD1 HNSCC cell lines by CRISPR Cas9. We are also in the process of evaluating the effect of the H3K36M overexpression in the wild-type NSD1, FaDu cells. Moreover, we want to further characterize the observed overexpression of LTR elements by analyzing the defective silencing and expression of retroviral elements in the presence or absence of NSD1.

[46] HISTONE DEACETYLASES PLAY A VITAL ROLE IN SYNCYTIOTROPHOBLAST FORMATION DURING PLACENTAL DEVELOPMENT

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INTRODUCTION: The multinucleated syncytiotrophoblast (ST) layer of the placenta has critical roles in pregnancy, including the regulation of nutrient and gas exchange between maternal and fetal blood and the production of various hormones. ST is maintained by differentiation (fusion) of underlying stem-like cytotrophoblast (CT) cells. Differentiation of CT cells into ST is associated with global repression of gene expression, and we hypothesize that this transcriptional repression is required for progression of differentiation. Histone deacetylases (HDACs) are a major family of transcriptional repressors, but their role in ST development is not well understood. Thus, the objective of this project is to determine whether HDAC-mediated transcriptional repression contributes to ST development.
Methods: Human trophoblast BeWo cells, which can be maintained as CT-like cells or induced to differentiate into ST by exposure to agents that increase intracellular cyclic adenosine monophosphate (cAMP), were treated with the global HDAC inhibitor, trichostatin A (TSA 1-20nM), or a class I HDAC inhibitor romidepsin (1-5nM). Cells were then induced to differentiate by exposure to 250 μM 8-bromo-cAMP for 48 h. To confirm the efficacy of the HDAC inhibitors, acetylation of histone H2B-K5, H3-K27 and H3-K14 was determined by western blotting. Subsequently, transcripts associated with syncytialization (ERVW-1, ERVFRD-1, CGB, HSD11B2) were analyzed by quantitative RT-PCR. The effect of HDAC inhibition on ST development was measured by immunofluorescence for E-cadherin and human chorionic gonadotropin.
Results: Differentiation of BeWo trophoblast cells was associated with increased levels of ST markers ERVW-1, ERVFRD-1, CGB, HSD11B2. Concomitantly, there was a progressive decrease in global histone H2B-K5, H3-K27, and H3-K14 acetylation, indicating elevated HDAC activity during ST formation. Following treatment with TSA or romidepsin, cells exhibited higher levels of histone H2B-K5, H3-K27, and H3-K14 acetylation, indicating reduced HDAC activity. Treatment of cells with TSA and romidepsin during exposure to differentiation conditions abrogated ERVW-1, ERVFRD-1, CGB, and HSD11B2 expression in a dose dependent fashion. Furthermore, exposure of cells to 20 nM TSA abrogated ST development by 75%, while treatment with 5 nM romidepsin inhibited ST development by 60%.
Conclusion: TSA and romidepsin were effective in inhibiting HDAC activity. Reduced HDAC activity prevented induction of various ST markers and abrogated ST development. Future studies will investigate the contribution of HDAC1 and HDAC2 to ST development.

[47] EPIGENETIC CHARACTERIZATION AND THERAPEUTIC TARGETING OF CANCERS HARBORING DYSFUNCTIONAL CTCF

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Each year in Canada, approximately 5000 women succumb to breast cancer, with over 90% of these deaths resulting from metastatic disease. By understanding molecular mechanisms that drive tumor progression, we aim to identify biomarkers predicting response to targeted therapies.
CTCF is a haploinsufficient tumor suppressor gene that acts to repress oncogenes and maintain the transcriptional activation of tumor suppressors. CTCF copy number loss is observed in roughly 50% of breast tumors and is likely an important driver of tumor progression, however, the functional impact of these modifications remains obscure. We used CRISPR/Cas9 to knockout an allele of CTCF in the normal mammary epithelial cell line MCF10A (MCF10A-CTCF$^{+/-}$) and found that a number of oncogenes were upregulated in the oncogenic and proinvasive "Epithelial to Mesenchymal Transition" (EMT) pathway. Consistent with this, MCF10A-CTCF$^{+/-}$ cells gained the capacity to invade through matrigel and were able to initiate small tumors in immunocompromised mice. Mechanistically, we find that knockdown of the EMT-promoting oncogene Snail, greatly reduces the ability of MCF10A-CTCF$^{+/-}$ cells to invade through matrigel.

Sequencing data highlighted the increase of PIK3-Akt pathway and TGFβ pathway activity in absence of CTCF and show that CTCF recruitment is lost/gained around key genes of these pathway. ChIP data show a diminution of H3K27me3 and an increase of H3K4me3 and H3K27ac around the promoter of the gene model SNAIL.
Recently, we reported that CTCF plays an integral role in the repair of DNA double strand breaks through the homologous recombination pathway. We have now discovered that MCF10A-CTCF$^{+/-}$ cells show sensitivity to cisplatin and PARP inhibitors. In collaboration with Dr. Mark Basik, we will employ patient derived xenograft models of breast cancer to test those carrying CTCF deletion for sensitivity to combination therapy with cisplatin and PARP inhibition.
Overall, this project will provide key insights regarding the processes that drive tumor growth and metastasis and highlight new therapeutic strategies to target poor-prognosis breast cancers.

[48] BORIS PROMOTES A SWITCH FROM A PROLIFERATIVE TO INVASIVE GENE SIGNATURE IN MELANOMA CELLS

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Background: Melanoma is one of the most aggressive cancers due to its tendency to metastasize. Melanoma progression is enabled by epithelial to mesenchymal transition (EMT)-like phenotype switching between a proliferative and invasive state. Transcriptional profiling of melanoma cell lines has identified gene signatures that correspond to these states. Brother of Regulator of Imprinted Sites (BORIS), also known as CTCF-Like, is a cancer testis antigen that becomes re-activated in melanoma. Expression of BORIS in cancer cells can alter gene expression, including that of genes involved in EMT.
Methods: To determine if BORIS expression alters the transcriptional signature of melanoma cells, we established doxycycline-inducible BORIS expression in a proliferative melanoma cell line and performed RNA-seq. In addition, we used ATAC-seq to determine if BORIS-induced changes in gene expression coincide with changes in chromatin accessibility.
Results: Analysis for differentially expressed genes (DEGs) revealed 2291 DEGs between BORIS and the controls of which 1490 were upregulated and 801 downregulated. To validate the RNA-seq data, DEGs were ranked by FDR and differential expression was confirmed by qPCR for every 300th upregulated and 200th downregulated gene. Gene set enrichment analysis showed a strong positive correlation with the “Hoek” and “Verfaillie” invasive gene signatures and a negative correlation with the proliferative gene signatures. In addition, the upregulation of invasive genes and down regulation of proliferative genes was validated by qPCR.
Ongoing experiments: We are currently assessing the effect of BORIS expression on the invasive potential of melanoma cells and are in the process of analyzing the ATAC-seq data.
Conclusion: These data show that BORIS expression alters the transcriptome of melanoma cells and indicate that BORIS promotes a switch from a proliferative to invasive gene signature. Ongoing work could elucidate how BORIS expression regulates the upregulation of invasive genes.

[49] NOVEL HIGH THROUGHPUT TOOLS FOR MEASURING EPIGENETIC MARKERS IN CFDNA AND CIRCULATING NUCLEOSOMES IN LIQUID BIOPSIES FROM AGING ADULTS AND CANCER PATIENTS

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Active Motif, Inc. Carlsbad CA

Active Motif has developed of a variety of assays which accommodate the high sample number throughput requirements of drug discovery and pharmaceutical companies interested in targeting epigenetic mechanisms. In recent years, liquid biopsies have shown promise for biomarker identification and treatment evaluation. Indeed, clinical researchers are using cell free DNA (cfDNA) to assess risk, disease progression, and treatment strategies and efficacy prospectively. To further support this effort, here we demonstrate the applicability of our novel high-throughput approaches to measure epigenetic alterations in human serum or plasma samples from cancer patients or from healthy young and old individuals.
Altered epigenetic regulation of gene expression is observed in most diseases, and in normal biological processes, such as aging. Aging-associated epigenetic changes such as global DNA hypomethylation, loss of histones, and alterations in specific histone marks, have been described in humans and other model organisms, and likely contribute to aging-associated cellular dysfunction. It is well established that methylation levels of Long Interspersed Nucleotide Element 1 (LINE-1) repeats serve as reliable proxy for assessing global DNA methylation in normal and disease conditions. Here, we show that aging-associated decreases in DNA methylation in LINE-1 repeats can be quantitated in circulating cell free DNA using the Global DNA Methylation – LINE-1 Kit, an ELISA-based assay for the detection and quantification of global DNA methylation (i.e., 5-methylcytosine (5-mC) levels).
In cancer, changes in DNA methylation patterns and its impact on cancer development and progression is well established. Immunohistochemical methods have been used to detect changes in global levels of histone PTMs in a several tumor type, and these changes have been linked to clinical outcome in numerous cancer types. We have developed a microsphere-based sandwich ELISA suspension array for the Luminex instrument platform for the simultaneous quantitative detection of histone H3 modifications. These beads can be combined with a single sample enabling evaluation of multiple histone modifications in lysates derived from culture cells and frozen tissues. We also demonstrate the applicability of a histone H3 bead-based multiplex ELISA, which simultaneously detects thirteen post-translational modifications to monitor changes in global histone modifications in human PBMCs. Using a

nucleosome-specific antibody, this assay can detect nucleosomes and H3 post-translational modifications in serum.

[50] IDENTIFICATION OF NOVEL DNA-BINDING PROTEINS INTERACTING WITH HP1 ISOFORMS THAT MEDIATE K9ME3-INDEPENDENT GENE SILENCING IN MOUSE ESCS.

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University of British Columbia

The three Heterochromatin Protein 1 (HP1) isoforms are generally thought to regulate transcription by binding to the covalent histone modification H3K9me3, causing chromatin compaction. However, studies of HP1 protein interactions have revealed that each isoform interacts with a wide range of other proteins, including many with putative DNA-binding activity, making it possible that HP1 isoforms can be recruited to genomic loci via these interactors, independently of H3K9me3. We aim to investigate five candidate HP1-interactors with putative DNA-binding activity for their function in HP1-mediated transcriptional repression; Ahdc1, Zfp462, Champ1, Zmym3 and Pogz. We hypothesize that each candidate is responsible for recruitment of HP1 proteins to distinct sets of genes in mouse embryonic stem cells (mESCs) to prevent transcription of such genes and maintain pluripotency. Using CRISPR/Cas9-mediated deletion in mESCs and transcriptome (RNAseq) analysis, we will study the effects on gene expression upon loss of each candidate. We will compare each expression profile to RNAseq data from mESCs lacking all three HP1 isoforms to identify target genes for HP1-mediated repression and use gene ontology analysis to determine whether candidates regulate distinct developmental programs. To establish a direct causal effect, we will perform rescue experiments in mutant mESCs and introduce point-mutated HP1 isoforms lacking the ability to either bind the candidate or binding H3K9me3, or point-mutated candidates lacking the HP1-interacting motif. These experiments will expand on the novel mechanism identified by Ostapcuk, et al. for HP1 protein recruitment, and might shed light on the process of cell differentiation and maintenance of pluripotency.

[51] INTEGRATIVE ANALYSIS OF 3D GENOME ARCHITECTURE IN GLIOBLASTOMA SELF-RENEWING CELLS IDENTIFIES CD276 A PUTATIVE IMMUNOTHERAPY TARGET.

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Recent advances in mapping distant DNA-DNA interactions have established that physical connectivity of the genome within the nucleus is an essential layer of epigenetic regulation. However, the pivotal role of 3D genome architecture remains inadequately explored in the context of cancer. Hypothesizing that erroneous DNA looping is an important part of glioblastoma (GBM) biology, we generated Hi-C contact maps to investigate DNA looping, domain structure, and compartmentalization in three patient-derived primary glioblastoma cultures. At sub-5-kb resolution, these contact maps constitute the highest resolution Hi-C datasets available for primary cultures from cancer patients. We further integrated this dataset with RNA-seq to assess transcriptional activity, CTCF ChIP-seq to identify sites of DNA loop anchors, and H3K27ac ChIP-seq to identify super-enhancers acting upon genes. Although many features were common between patients, significant patient-specific differences in genome architecture were evident. Genes with patient-specific loops – especially those forming loops to super-enhancers – were significantly more likely to be up-regulated, opening the possibility to future therapies targeting loop architecture to downregulate gene activity. Patient-specific differences in compartmentalization and looping at key stem cell regulators SOX2 and ASCL1 manifested in differential transcription of these genes between patients. We also identified a patient-specific chromosomal rearrangement that enables looping between the JAK1 locus and two super-enhancers >100 Mb distant in the unrearranged genome. In contrast, by scanning for regions of similarity among GBM samples, we identified regions of GBM-specific open compartmentalization with enrichment for immune response genes. This led us to led us to investigate the therapeutically-targetable CD276. We demonstrate that CD276 is highly expressed in all three patient cultures and that treatment with a CD276-targeting immunotherapeutic, m276-PBD, significantly impairs viability and self-renewal of cultured GBM cells. Altogether, this work integrates the emerging importance of 3D genome architecture with fundamentals of epigenetic regulation and cancer biology.

[52] PRC2-DEPENDENT TISSUE-SPECIFIC 3D CHROMATIN ARCHITECTURE REVEALS A MECHANISM FOR THE ATYPICAL CONTRIBUTION OF PRC2 IN GENE ACTIVATION

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Embryonic development relies on coordinated expression of developmental genes. Preventing inappropriate gene expression in time and space is as fundamental as triggering the activation of tissue/cell-type specific factors at the correct developmental stage and in the correct cell population(s). Here, we investigate the role of Polycomb group proteins in gene regulation, with particular emphasis on their contribution to 3-Dimensional (3D)

chromatin organization. Using the regulation of HoxA genes in the developing limb as a model system, our analyses reveal the requirement of Polycomb Repressive Complex 2 (PRC2) for long-range contacts between silent HoxA genes and PRC-bound loci located in the regulatory landscapes (+/- 2Mbs) that flank the gene cluster. We also found that the differences in chromatin architecture that distinguish the proximal limb from the distal limb rely on PRC2 function. Interestingly, our data provide evidence that PRC2-dependent chromatin architecture has a dual impact on enhance-promoter physical proximity. For instance, in the proximal limb, it promotes contacts between proximal limb enhancers and Hoxa9-Hoxa11, whose function is required for forearm patterning, while restraining contacts between Hoxa13 and the distal limb enhancers. Based on these results, we propose that the non-conventional PRC2 function in promoting gene transcription relies, at least in part, on modulations of the 3D chromatin architecture mediated by PRC2.

[53] THE CARTAGENE PLATFORM: A PUBLIC RESOURCE FOR EPIGENOMIC STUDIES

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Genetic and epigenetic studies have greatly contributed to the identification of risk factors for chronic diseases. The accepted hypothesis is that the interactions between genetic and environmental exposures lead to modifications in gene expression, which play a role in disease outcomes. Supporting the scientific community in identifying the determinants of chronic diseases of environmental and/or genetic origin is CARTaGENE's (CaG, https://www.cartagene.qc.ca) mission.
CaG is both a population-based biobank and the largest ongoing prospective health study of men and women in Quebec. CaG recruited 43 000 individuals, aged 40-69 years, representing six metropolitan regions and collected detailed lifestyle, health and medical data on these individuals. CaG also gathered detailed physical measurements, clinical and biochemical data as well as nutrition and environmental data. More than 4000 unique variables are available for each participant.
CaG generated genotyping, sequencing, and epigenomic data using collected biological samples. Current epigenomics and genetic data include whole genome genotyping (n=12,000), exome sequencing (n=198), RNA sequencing (n=911), methylc-capture sequencing (n=137) and Infinium HumanMethylation450K (n=94). The genotyping of the whole cohort is underway and additional data is periodically available. Blood samples are available for over 30,000 participants including repeat samples (>1500). Tempus tubes for ARN extractions are also available (>8500). Recontact of participants to collect additional data and biosamples is possible.
CaG databases are linked to administrative health databases (Med-Echo), as well as environmental (CANUE) and genealogical databases (Balsac). CaG is fully integrated with the Canadian Partnership for Tomorrow Project, which represents 300,000 participants and more than 150,000 biological samples available for health research.
One of the unique features of CaG is the participant's broad consent that allows sharing of data and biosamples for health-related research projects. More than 40 research projects have involved CaG's data including projects on the genetics of breast cancer, cardiometabolic traits, and rheumatic diseases. CaG is a public resource available to researchers worldwide and offering powerful tools for health research.

[54] HIGH-FAT DIET PROPELS PROSTATE CANCER BY REWIRING THE METABOLOME AND AMPLIFYING THE MYC PROGRAM

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Systemic metabolic alterations associated with increased consumption of saturated fat and obesity are linked with increased risk of prostate cancer progression and mortality, but the molecular underpinnings of this association are poorly understood. Furthermore, the mechanisms by which metabolic rewiring alters the prostate cancer epigenome, the effector arm of intra- and extra-cellular signals, is equally nebulous. Here, we demonstrate, in a murine prostate cancer model, that high-fat diet (HFD) enhances the MYC transcriptional program through metabolic alterations that favour histone H4K20 hypomethylation

at the promoter regions of MYC regulated genes, leading to a HFD-dependent phenotype characterized by increased cellular proliferation and tumour burden. Importantly, these results are recapitulated in prostate cancer patients, where increased saturated fat intake (SFI), but not monounsaturated or polyunsaturated fat intake, is also associated with an enhanced MYC transcriptional signature. Additionally, the SFI-induced MYC signature independently predicts prostate cancer progression and death. Finally, a dietary intervention consisting of switching from a high-fat to control diet, greatly attenuates the MYC transcriptional program. Our findings suggest that in primary prostate cancer, dietary fat intake contributes to tumour progression by mimicking MYC over expression, setting the stage for therapeutic approaches involving changes to the diet.

[55] IDENTIFICATION OF EPIGENOMIC SIGNATURES IN SPERM ASSOCIATED WITH BODY MASS INDEX AND FERTILITY STATUS

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Introduction: Sperm counts in men have been declining at an alarming rate with counts being half of what they were 40 years ago[1,2]. This decline may be attributable to >50% of the population being overweight. In mice and men, the sperm epigenome including DNA and histone methylation, has been linked to responses to diet, body mass index (BMI), fertility and altered reproductive outcomes. Previously we showed that changes in the enrichment of histone methylation in sperm are associated with poor reproductive outcomes[3,4]. These studies suggest that the sperm epigenome has the potential to be used clinically to assess fertility. Our objective was to profile histone H3 tri-methylation on lysine 4 (H3K4me3) in sperm from men with differential BMI status (normal vs high) in couples seeking fertility treatment at the CReATe clinic. **Methods**: We recruited study participants (n=137) and collected BMI, dietary intake, folate parameters (folate, vitamin B12, homocysteine), vitamin D levels and lifestyle information. Semen quality was analyzed by standard clinical approaches. Men selected for analysis were of either a normal BMI (BMI <25kg/m^2) or overweight and obese (BMI >25 kg/m^2). Men were excluded if they had a DNA fragmentation index >30, if they were older than 50, smoked and had the TT genotype for the C677T SNP of the MTHFR enzyme. ChIP-seq targeting H3K4me3 was performed on individual patients with a normal (n=24) or increased BMI (n=24). **Results**: All men were folate sufficient (320 – 1090 nmol/L), with 62% having high levels of RBC folate at >1090 nmol/L. We did not detect significant differences between the folate status of men with a normal (n=52) and increased BMI (n=85). Unlike folate, there were men with low vitamin D status; 20.6% of the participants were deficient (<30 nmol/L) and 32% were insufficient (30 to <50 nmol/L). Bioinformatic analysis of ChIP-seq data was performed using the Bioconductor package csaw and identified 9972 regions (150 to 2450bp) with a differential enrichment in H3K4me3 between men with a normal BMI and men with an elevated BMI. Interestingly, regions gaining H3K4me3 in overweight and obese men were located mostly at promoters while regions losing H3K4me3 were found predominantly in introns and intergenic regions. A gene ontology analysis on the differentially H3K4me3-enriched regions located at promoters highlighted the role of the marked genes in cell cycle, spermatogenesis, in utero development and fat cell differentiation. Our long-term goal is to identify and link epigenomic signatures associated with BMI, fertility and clinical outcomes (e.g. embryo quality, pregnancy). **Conclusions**: Lifestyle factors may play a crucial role in the programming of the sperm epigenome and thus influence male fertility and clinical outcomes.

[56] CPG METHYLATION, A PARENT-OF-ORIGIN EFFECT FOR MATERNAL BIASED TRANSMISSION OF CONGENITAL MYOTONIC DYSTROPHY

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CTG repeat expansions in DMPK cause myotonic dystrophy (DM1) with a continuum of severity and ages-of-onset. Congenital DM1 (CDM1), the most severe form, presents distinct clinical features, large expansions, and almost exclusive maternal transmission. The correlation between CDM1 and expansion size is not absolute, suggesting the contribution of other factors. We determined CpG methylation flanking the CTG repeat in 79 blood samples from 20 CDM1 individuals, 21, 27, and 11 non-CDM1 individuals with maternal, paternal, and unknown inheritance, and collections of maternally- and paternally-derived chorionic villus samples (7 CVS) and 4 hESCs. All but two CDM1 individuals showed high levels of methylation

upstream and downstream of the repeat, greater than non-CDM1 individuals (p=7.04958E-12). Most non-CDM1 individuals were devoid of methylation, where one in six showed downstream methylation. Only two maternally-derived non-CDM1 individuals showed upstream methylation, and these were childhood onset, suggesting a continuum of methylation with age-of-onset. Only maternally-derived hESCs and CVS showed upstream methylation. In contrast, paternally-derived samples (27 blood samples, 3 CVS, and 2 hESCs), never showed upstream methylation. CTG tract length did not strictly correlate with CDM1 or with methylation. Thus, CpG methylation patterns flanking the CTG repeat are stronger indicators of CDM1 than repeat size. Spermatogonia with upstream methylation may not survive due to methylation-induced reduced expression of the adjacent SIX5, thereby protecting DM1 fathers from having CDM1 children. Thus, DMPK methylation may account for the maternal bias for CDM1 transmission, larger maternal CTG expansions, age-of-onset, clinical continuum, and may serve as a diagnostic indicator.

[57] AGE EFFECT ON THE REGENERATIVE CAPACITY OF SKELETAL MUSCLE STEM CELLS

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Adult stem cells are the driver of tissue regeneration. In skeletal muscle, a rare population of Muscle Stem Cells (MuSCs), also called Satellite Cells, maintains lifelong regenerative capacity of muscle. Satellite cells are situated in a defined position along the periphery of muscle fiber called their “niche”, where they are in constant cross-talk with neighboring cells through various signaling pathways. Upon a signal from their niche, satellite cells have the ability to become activated to either maintain the satellite cell pool or differentiate to repair damaged tissue. However, aging has a detrimental effect on satellite cells’ regenerative ability. The extent to which the age-related defect in satellite cell function is cell intrinsic or is induced by factors of the aging niche is poorly understood.
Epigenetic mechanisms play a key role in the aging phenotype of adult tissues. Due to the plasticity of epigenetic marks, we hypothesize that the niche is capable of reprogramming the transcriptome of satellite cells through modification of the epigenome. In order to determine how the epigenetic landscape is altered during aging, we analyze muscle stem cells within their niche in young and old mice by ChIP (Chromatin Immunoprecipitation) sequencing for key histone marks of active and repressed genes.
To identify which age-related disease-causing changes are reversible, we perform allogeneic muscle stem cell transplantation from old donors to young recipient mice. By reisolation of transplanted donor cells, we found that old satellite cells can repopulate the skeletal muscle of young irradiated immunocompromised mice. Importantly, donor stem cells home to the correct sub laminar niche location. To determine the transcriptomic and epigenomic alterations associated with the aging niche on the regenerative capacity of muscle stem cells, we use single cell technologies such as Switching Mechanism at 5’ End of RNA Template (SMART) to map alterations in the pattern of key regulatory histones and the resulting effects on transcriptome by RNA-Seq.
Comparative analysis of gene expression and epigenetic alterations before and after stem cell transplantation will identify niche-induced changes in the transcriptome. Importantly, combining epigenetic and transcriptomic data will identify reversible age-related pathways that can be used as potential epigenetic drug targets in patients with age-related muscle-wasting diseases.

[58] CHARACTERIZATION OF A NEW LNCRNA CANDIDATE TO (DE)REGULATE HOX CLUSTERS IN AGGRESSIVE GLIOMA

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Aggressive gliomas, that are characterized by IDH1 gene wild-type status (IDHwt), are highly recurrent brain tumors. Therapeutic resistance and tumor relapse is believed to rely on a subpopulation of cells, the so-called Glioma-Initiating Cells (GICs). Determining the molecular bases of GIC pathological features represent thus a major issue to improve our understanding of glioma biology.
In a founding project aiming to investigate for the role of epigenetics deregulations, and more specifically those affecting bivalent chromatin, we highlighted that alteration of the four HOX genes clusters is a hallmark of aggressive gliomas. By conducting exhaustive molecular analyses on clinically well characterized glioma tumoral samples and GIC lines, we indeed observed that profound molecular defects, associating ectopic expression, global DNA hypermethylation and a massive alteration of the H3K27me3 signature, affect the four HOX clusters as a whole in IDHwt tumors and GIC samples.
The 39 coding HOX genes play a key role in development, particularly in the organization of the anteroposterior axis. Consistently, their deregulation contributes to the carcinogenesis process. In normal conditions, the regulation of HOX clusters relies on chromatin remodeling and involves ribonucleic actors such as HOTAIR and HOTTIP. These two transcripts are antisense to HOX coding genes and control HOX loci during the development by recruiting the Polycomb and Trithorax complexes, respectively, contributing for the activation or the repression either in cis or trans of HOX genes.
In an effort to determine the molecular bases of this concerted deregulation at HOX clusters, we precisely identified a new non-coding antisense RNA, called HOXA-AS2, at the HOXA cluster. Specifically, we evidenced that this transcript is significantly and specifically overexpressed in IDHwt tumors and GICs, where its overexpression highly correlate with those of HOX-coding-transcripts from the 4 clusters. In addition, sh-RNA-based depletion of HOXA-AS2 drastically affects GIC phenotypes, both at the cellular and transcriptional levels.

Taken together, our data support a model whereby deregulation and overexpression of HOXA-AS2 is a founder event that lead to the chromatin alteration and overexpression of target genes, including HOX genes, which in turn will contribute to glioma initiation and progression. We are currently testing this original "two-step" model by using complementary descriptive and functional approaches conducted on well-characterized GIC and H9-derived Neural Stem Cell lines.
Completion of this project will represent a step forward to our understanding of lncRNA mechanism of action in pathological context. In addition, given that HOX loci deregulation is a hallmark of many cancers, we anticipate that main lessons of our project will be of interest to several malignancies. More specifically, our project will pave the way to develop strategies to eliminate glioma-initiating stem cells.

[59] EPIGENETIC REGULATOR MLL4 COMPLEX IN HEPATIC STEATOSIS

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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. PPARg2 is a hepatic steatotic transcription factor induced by overnutrition. Here, we report that the histone H3 lysine 4 methyltransferase MLL4 directs overnutrition-induced murine steatosis via its coactivator function for PPARg2. We demonstrate that overnutrition facilitates the recruitment of MLL4 to steatotic target genes of PPARg2 and their transactivation via H3 lysine 4 methylation because PPARg2 phosphorylated by overnutrition-activated ABL1 kinase shows enhanced interaction with MLL4. We further show that Pparg2 (encoding PPARg2) is also a hepatic target gene of ABL1-PPARg2-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-PPARg2-MLL4, which may serve as a target of anti-steatosis drug development.

[60] PREIMPLANTATION ALCOHOL EXPOSURE LEADS TO DNA METHYLATION DYSREGULATIONS IN THE EMBRYO THAT PERSIST THROUGHOUT THE GESTATION AND INDUCE COGNITIVE IMPAIRMENTS.

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Prenatal alcohol exposure (PAE) is known to alter epigenetic profiles in cells during brain development and be part of the molecular basis underpinning Fetal Alcohol Spectrum Disorder (FASD) etiology. However, the consequences of a PAE during very early embryonic life (preimplantation period) on the future epigenetic landscape of embryonic tissues remain unknown.
Hypothesis: Our research hypothesis is that a PAE during preimplantation will initiate DNA methylation dysregulations that will later be observable in the developing conceptus. We believe that these original epigenetic alterations will be perpetuated and amplified in the developing brain and will induce cognitive impairments after birth.
Objective: Our objective was to identify DNA methylation dysregulations in the forebrain of mid-gestation and late-gestation mouse embryos initiated by early PAE. Furthermore, wanted to uncover how early embryonic PAE leads to cognitive impairments in offspring.
Method: To test this, we used our preclinical model of early alcohol exposure by injecting exposing pregnant females to binge-like concentration of ethanol at E2.5 (8-cell stage). We collected FASD (ethanol) and control (saline) E10.5 and E18.5 embryos, and then established genome-wide quantitative DNA methylation profiles of forebrains. Two methods were used, at E10.5 we used Reduced Representation Bisulfite Sequencing, and at E18.5 we used a Methyl-Seq enrichment workflow (Agilent). Regions of a 100bp with more than ±20% difference in methylation between the controls and ethanol-exposed samples were considered as differentially methylated regions (DMR). To test cognitive capacity, we performed three behavioural tests: Open field, 3-chamber maze and novel object recognition with prenatally exposed pups at P40.
Results: We respectively observed 686 and 314 DMRs at E10.5 and E18.5 with a majority of DMRs being hypermethylated at both developmental time points. Gene ontology analysis revealed enrichment for neuronal and synapse function or differentiation a both time points, suggesting that the dysregulation of DNA methylation associated with these processes persist through gestation. Interestingly, when we did sex-specific analyses, we found that male embryos a higher number of DMRs compared to females (E10.5: 1677 vs 417; E18.5: 604 vs 158). Behavioural assessment revealed that the exposure did not alter anxiety or locomotion status (open field test) but showed impairments in social interaction (3-chamber maze), spatial recognition and object recognition (novel object recognition test).
Conclusion: Our study clearly reveals that an early acute alcohol exposure during the epigenetic reprogramming wave occurring in early embryos triggers long-lasting DNA methylation perturbations in the embryo, which leads to abnormal cognitive function in the developing offspring.

[61] NUCLEOSOME ACETYLATION SELECTIVELY AFFECTS NUCLEOSOME METHYLATION

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Posttranslational modification of histones plays a critical role in regulation of gene expression. These modifications include methylation and acetylation and work in combination to establish transcriptionally active or repressive chromatin states. Histone methyltransferases (HMTs) often have variable levels of activity in vitro depending on the form of substrate used. For example, certain HMTs prefer nucleosomes extracted from human or chicken cells as substrate compared to recombinant nucleosomes reconstituted from bacterially produced histones. We hypothesized that pre-existing histone modifications in the extracted nucleosomes can affect the efficiency of catalysis by HMTs suggesting functional cross-talk between histone modifying enzymes within a complex network of interdependent activities. Here, we systematically investigated the effect of nucleosome acetylation by p300, GCN5L2 and MYST1 (MOF) on histone 3 lysine 4 (H3K4), H3K9 and H4K20 methylation of nucleosomes by nine HMTs (MLL1, MLL3, SET1B, G9a, SETDB1, SUV39H1, SUV39H2, SUV420H1 and SUV420H2). Our full kinetic characterization data indicate that site specific acetylation of nucleosomal histones by specific acetyltransferases can create nucleosomes that are better substrates for specific HMTs. This includes significant increases in catalytic efficiency of SETDB1. We suggest that such acetyltransferase specificity is possibly resulting in better accessibility of histone marks for methylation.

[62] KINETIC CHARACTERIZATION OF HUMAN HISTONE H4 LYSINE 20 METHYLTRANSFERASES SETD8 AND SUV420H1/2

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SETD8/SET8/Pr-SET7/KMT5A, a member of the SET domain-containing methyl-transferase, is the only lysine methyltransferase known to monomethylate lysine 20 of histone H4 (H4K20) in vivo. Because mono-methylation of H4K20 regulates diverse biological processes, SETD8 has been pursued as a potential therapeutic target. In this study, we reported full kinetic characterization of SETD8, and provide robust in vitro assays suitable for screening in a 384-well format. Although active with histone 4 peptide H4 1-24 as a substrate, SETD8 showed higher catalytic efficiencies with nucleosomes. Recombinant nucleosome is the preferred substrate compared to native nucleosomes from chicken or human. Analysis of the product specificity confirmed that SETD8 is a monomethylase. The Mono-methylated recombinant nucleosome generated by SETD8 is the preferential substrate for lysine methyltransferases SUV420H1 and SUV420H2. Thus, H4K20 methylation has been proposed to be an important mark to the chromatin and thereby regulating transcriptions.

[63] ROBUST JOINT SCORE TESTS IN THE APPLICATION OF DNA METHYLATION DATA ANALYSIS

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Background: Recently differential variability has been showed to be valuable in evaluating the association of DNA methylation to the risks of complex human diseases. The statistical tests based on both differential methylation level and differential variability can be more powerful than those based only on differential methylation level. Anh and Wang (2013) proposed a joint score test (AW) to simultaneously detect for differential methylation and differential variability. However, AW's method seems to be quite conservative and has not been fully compared with existing joint tests.
Results: We proposed three improved joint score tests, namely iAW.Lev, iAW.BF, and iAW.TM, and have made extensive comparisons with the joint likelihood ratio test (jointLRT), the Kolmogorov-Smirnov (KS) test, and the AW test. Systematic simulation studies showed that: 1) the three improved tests performed better (i.e., having larger power, while keeping nominal Type I error rates) than the other three tests for data with outliers and having different variances between cases and controls; 2) for data from normal distributions, the three improved tests had slightly lower power than jointLRT and AW. The analyses of two Illumina HumanMethylation27 data sets GSE37020 and GSE20080 and one Illumina Infinium MethylationEPIC data set GSE107080 demonstrated that three improved tests had higher true validation rates than those from jointLRT, KS, and AW.
Conclusions: The three proposed joint score tests are robust against the violation of normality assumption and presence of outlying observations in comparison with other three existing tests. Among the three proposed tests, iAW.BF seems to be the most robust and effective one for all simulated scenarios and also in real data analyses.

[64] CHOOSING PANELS OF GENOMICS ASSAYS USING SUBMODULAR OPTIMIZATION

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Genomics assays such as ChIP-seq, DNase-seq and RNA-seq can measure a wide variety of types of DNA activity, but the cost of these assays limits their application. In principle, to fully characterize a cell type, one would like to perform every possible type of assay, including ChIP-seq targeting variety of histone modifications and dozens of transcription factors, as well as several variants of chromatin accessibility and RNA-seq. However, at current sequencing prices, performing a single genomics assay with reasonable sequencing depth costs on the order of $400.
In practice, many pairs of assay types yield redundant information. For example, the histone modification H3K36me3 primarily marks gene bodies, which are also transcribed and therefore measured by RNA-seq. This redundancy among assay types suggests that a carefully chosen panel of assays is likely to produce most of the information that would be obtained by performing all assays.

Consequently, selecting a small panel of assays to perform on each cell type of interest—a problem we call assay panel selection—is a key step in any genomics project. To our knowledge, there has been little discussion in the literature of how to choose such a panel.

In this work, we propose a principled computational method for assay panel selection, Submodular Selection of Assays (SSA). Qualitatively, the method aims to identify, on the basis of existing data sets, assay types that yield complementary views of the genome. Our solution is composed of two parts: a quality function that defines the quality of a panel, and an optimization algorithm that efficiently finds a panel that scores highly according to the quality function. We propose a quality function that measures what fraction of the information available in the full set of assay types is contained within the panel. A simple approach to selecting a panel of assays would evaluate the objective function for every possible subset of assays and then choose the highest-quality subset. Unfortunately, 216 existing assay types yield $2^{216} \approx 10^{65}$ possible panels of assays, so this approach is not feasible in practice. Fortunately, our quality function has the mathematical property of submodularity, which allows us to apply existing fast algorithms for optimizing such functions.

We compare our method to alternative strategies according to how the resulting panels perform on three downstream applications: (1) the accuracy with which the panel can be used to impute the results of assays not included in the panel; (2) the accuracy with which the panel can be used to detect functional elements such as promoters and enhancers; and (3) the quality of a whole-genome annotation produced using the panel.

[65] THE EFFECTS OF A FOLATE DEFICIENCY ON THE SPERM EPIGENOME AND THE IMPLICATIONS IN EMBRYO DEVELOPMENT

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INTRODUCTION Congenital anomalies affect 3% of all births and are responsible every year for the death of over 300 000 infants aged 4 weeks and under (WHO, 2016). Children born from populations of low socio-economic backgrounds are at a higher risk of developing a complex disease and the health disparities that exist within these vulnerable populations have increased over the past decade (WHO, 2016). A father's environmental exposure to toxicants and poor diet influence disease transmission across generations, potentially through epigenetic inheritance (Lambrot et al., 2015 & Li et al., 2017 & Carone et al., 2010). However, the consequences of environmental stressors on the sperm epigenome and the paternal contributions towards embryonic development need to be elucidated. We aim to better understand the mechanisms governing paternal epigenetic inheritance by studying how a folate deficiency in male mice affects their sperm epigenome and how a dietary stressor alters epigenetically sensitive regions in the sperm chromatin.

METHODS To investigate if a paternal folate deficiency outside periods of epigenetic reprogramming altered the sperm epigenome, we fed wildtype (WT) C57BL/6 males a folate sufficient (FS, 2.0 mg/kg) or folate deficient (FD, 0.3 mg/kg) diet beginning at weaning for a full spermatogenic cycle. In order to determine whether there can be cumulative damages to the sperm epigenome and on offspring health, we also fed a FS or FD diet to a transgenic (TG) mouse model overexpressing the lysine-specific histone demethylase KDM1A in sperm. This transgenic model has an altered sperm epigenome with altered histone methylation at lysine 4 on histone H3. The WT and TG male mice fed either a FS or FD diet were then bred to WT C57BL/6 females on a regular diet. Pregnancy losses and a quantitative skeletal analysis were assessed on embryonic day 18.5 mice (7 – 10 litters per experimental group, 2 fetuses randomly selected per litter) to determine early pregnancy outcomes and to evaluate for abnormalities in the offspring. To quantitate the epigenetic consequences of a folate deficiency beginning at weaning on the sperm epigenome and the effects of adding an environmental perturbation of a FD diet to TG males with an already eroded sperm epigenome, we performed ChIP-sequencing for H3K4me3 on the sperm of the adult sires (n = 5 males per group) followed by analysis using the Bioconductor package csaw (Lun et al., 2016).

RESULTS Paternal folate deficiency in WT and TG males was associated with an increase number of pre-implantation losses. Skeletal analysis at embryonic day E18.5 revealed a significant increase in severe abnormalities in the offspring sired by FD TG. These abnormalities included craniofacial and spinal defects as well as pronounced developmental delays. In addition, preliminary analysis suggests that sires on the FD diet have altered H3K4me3 enrichment in their sperm. Analysis is ongoing.

CONCLUSION This work suggests that the post-natal period through to adulthood is also sensitive to environmental programming by diet and that different epigenetic stressors can carry a cumulative effect on the sperm epigenome and lead to greater number of abnormalities in the offspring. Our work will determine how environmental exposures affect the sperm epigenome. Acquiring a better understanding of how environmental stressors alter epigenetically sensitive regions in the sperm and how this is reflected in the developing offspring, will contribute to the broader understanding of how paternal lifestyles can influence embryonic development and offspring health.

[66] IMPACT OF HIGH FOLATE INTAKE DURING PREGNANCY ON PLACENTAL GENE EXPRESSION AND EPIGENETICS IN MICE

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Folate, an important B vitamin, is essential for reproduction and development. Folate metabolism is required for the transfer of one-carbon units in several key biosynthetic pathways, including synthesis of S-adenosylmethionine (SAM), the universal methyl donor. Since the mid-1990s, grains and cereals have been fortified with folic acid (FA), the synthetic form of folate, in over fifty countries, to reduce the incidence of neural tube defects. However, some women are consuming multivitamins with high

doses of FA (5mg/day, ~10 times higher than recommended dose of 0.4mg/day) which can lead to high serum levels of unmetabolized FA, raising concerns about the potential detrimental effects of high FA intake during pregnancy. To further study the impact of maternal high folate intake during pregnancy on offspring development, wild-type female mice were placed on a control diet (CD; recommended level of folate for rodents) or FASD (10-fold higher folate than recommended level) for 5 weeks prior to mating and maintained on the same diet during pregnancy and lactation. Embryonic growth delay and short-term memory impairment in 3-week-old offspring were observed. Mothers and offspring had reduced hepatic immunoreactive protein levels of methylenetetrahydrofolate reductase (MTHFR), a key enzyme in the folate pathway, suggesting they have a pseudo-MTHFR deficiency. MTHFR generates the folate derivative for SAM synthesis.
Since the survival and growth of the fetus are critically dependent on the placenta, the altered development of offspring from FASD mothers may be initially due to changes in placenta. We therefore measured the expression of genes involved in folate metabolism and methylation homeostasis and observed increased mRNA levels of Dnmt3a and folate receptors Folr1 and Slc19a1 due to FASD. In addition, there was a trend for increased SAM in the placenta of the FASD group. To further study gene expression differences in the placenta due to diet, microarray analysis was performed. Genes involved in extracellular matrix stabilization, the Wnt pathway and insulin-like growth factor-dependent pathways were differently expressed due to diet. qRT-PCR further showed that FASD diet led to reduced mRNA levels of Cnn1, Col5a1, Pitx2, Igfbp3 and Sfrp2. DNA methylation assays will be performed as appropriate. These findings show that high folate intake during pregnancy can lead to pseudo-MTHFR deficiency, which combined with placental changes may contribute to deleterious effects of high folate intake during pregnancy.

[67] DNA METHYLATION IN AGRP NEURONS REGULATES VOLUNTARY EXERCISE BEHAVIOUR

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Background: Voluntary exercise is an important component of metabolic health and energy balance and shows substantial inter-individual variation. Adult propensity for voluntary exercise is subject to developmental programming by early-life environment. Because Agouti-Related Peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARH) are key mediators of energy balance, inter-individual variation in energy intake and expenditure may be influenced by epigenetic alterations in these neurons.
Objective: To determine whether DNA methylation in AgRP neurons affects energy balance.
Methods: We generated mice lacking the de-novo methyltransferase gene Dnmt3a specifically in AgRP neurons (AgRP-Cre; Dnmt3a$^{F/F}$) and subjected them to a panel of metabolic and behavioural tests including indirect calorimetry, voluntary wheel running, and metabolic treadmill testing. We used whole-genome bisulfite sequencing (WGBS) and RNA-Seq on FACS-purified ARH neurons to identify induced alterations in transcription and methylation in knockout (F/F) mice. To localize methylation changes to individual cell types, we developed a novel approach to 'digitally dissect' WGBS data.
Results: Mice lacking Dnmt3a in AgRP neurons exhibited a sedentary phenotype in adulthood, characterized by increased adiposity and reduced voluntary exercise. This was associated with defects in AgRP neural projections and local GABAergic signaling. WGBS analysis revealed over 16,000 hypomethylated differentially methylated regions (DMRs) in ARH neurons from F/F mice; surprisingly, however, we also found nearly 60,000 hypermethylated DMRs. These epigenetic changes were associated with widespread transcriptional alterations, with differentially expressed genes showing enrichment for gene ontology terms related to GABAergic signaling and neural development. To overcome the challenge of cell type heterogeneity in our samples, we applied a novel 'digital dissection' approach to our WGBS data. This led us to identify Bmp7 upregulation and promoter hypomethylation in AgRP neurons, along with hypermethylation of genes downstream of Bmp receptor signaling in non-AgRP neurons, as a key potential signaling pathway involved in the neuroepigenetic phenotype of knockout mice.
Interpretation: Detailed studies of early environmental influences on adult energy balance have demonstrated developmental plasticity of voluntary exercise behaviour, but not food intake; epigenetic mechanisms are almost certainly involved in these effects. Consistent with this, our current data indicate that DNA methylation in AgRP neurons regulates adult voluntary exercise behavior, suggesting that individual variation in propensity for exercise is regulated by epigenetic mechanisms in the central nervous system.

[68] SPT5 PROMOTES EFFICIENT TRANSCRIPTION TERMINATION TO ANTAGONIZE HETEROCHROMATIN

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Aberrant transcription by RNA polymerase II (RNAPII) is implicated in numerous human diseases, including cancer, cardiac hypertrophy and HIV. A detailed characterization of the function and regulation of RNAPII is necessary to understand diseases associated with aberrant transcription. Processivity of RNAPII is regulated by Spt5, an essential elongation factor that couples transcription to various aspects of RNA processing, DNA repair and chromatin modifications. The C-terminal domain (CTD) is required for full Spt5 function. A novel role for the CTD in regulating transcription termination was recently identified, with phosphomimetic threonine-to-glutamate (T1E) mutations causing transcriptional read-through beyond the termination site. The mechanisms through which the CTD couples elongation and

termination to other processes are not understood. To address this gap, we completed a genetic screen to identify mutations that enhance phenotypes of Spt5 CTD mutants as a means to functionally characterize the Spt5 CTD. The fission yeast Schizosaccharomyces pombe was used as a model, as its epigenome and transcriptional regulation is similar to that of mammalian cells.

The screen uncovered genetic interactions between Spt5 mutants and proteins associated with heterochromatin boundaries. Defective boundaries can lead to an overabundance of heterochromatin and aberrant gene silencing, phenotypes associated with certain cancers. Thus, I hypothesized that the CTD is required for heterochromatin boundary function and predicted that the Spt5 mutant lacking the CTD (Spt5ΔC) would exhibit increased heterochromatin. Defective termination similar to that seen in Spt5-T1E mutants has been linked to increased heterochromatin. The CTD may regulate heterochromatin by recruiting termination factors in a phosphorylation-dependent manner.

Methylation of histone H3 on lysine 9 (H3K9me) is a conserved marker of heterochromatin. Anti-H3K9me chromatin immunoprecipitation (ChIP) in Spt5ΔC cells established that heterochromatin had spread past the boundaries. Heterochromatin-proximal reporter gene assays were used to assess boundary function and indicated that the increase in H3K9me was indeed associated with decreased gene expression. Thus, the Spt5 CTD antagonizes heterochromatin spreading. As transcriptional read-through as seen in Spt5-T1E cells has been linked to increased H3K9me, we proposed that the unphosphorylated CTD recruits an unidentified termination factor to prevent heterochromatin spreading. ChIP experiments in mutants with defective termination exhibited increased H3K9me. Additional experiments will determine if the Spt5 CTD functions to recruit these termination factors. We will establish whether the CTD directly interacts with the candidates. This project will provide mechanistic insight into how the Spt5 CTD regulates heterochromatin and prevents aberrant gene silencing.

[69] CTCF/COHESIN BINDING SITES ORGANIZED AS CLUSTERS AT BOUNDARIES OF TOPOLOGICALLY ASSOCIATED DOMAIN

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The genome is partitioned into different compartments subdivided into topologically associated domains (TADs) that discriminate active from repressive domains. CTCF, cohesin (RAD21, SMC1 and SMC3), ZNF143 and the YY1 transcription factors were previously reported to preferentially bind chromatin at TAD boundaries delineating anchors of chromatin interactions. We assessed if these transcription factors are enriched within clusters of cis-regulatory elements (COREs) at TAD boundaries based on their ChIP-Seq signal intensity in both GM12878 and K562 cell lines. CTCF and RAD21 are preferential enriched within COREs rather than individual CREs restricted to TAD boundaries (FC at COREs more than 1.5 times the FC at Ind. CREs). No enrichment over COREs at TAD-boundaries is seen for ZNF143 and YY1, or any of the 82 and 94 additional transcription factors with ChIP-seq data in GM12878 and K562 cells, respectively. Together, this argues that CTCF and cohesin behave differently from all other transcription factors at TAD-boundaries, mapping to COREs as opposed to individual CREs. Furthermore, CTCF and cohesin bind at TAD-boundary COREs with higher intensity than at intra-TAD COREs in both GM12878 and K562 cell lines (FC>2, FDR<0.001 for CTCF and RAD21; FC>1.7, FDR<0.001 for SMC3). ZNF143 also preferentially occupies TAD-boundary COREs as opposed to intra-TAD COREs but only in K562 cells (FC=1.42, FDR < 0.001). We observed lesser differences in the binding intensity of YY1 at TAD-boundary COREs versus intra-TAD COREs in either GM12878 and K562 cell lines (FC<1.25 in both cell lines).

The enrichment of CTCF and the cohesin complex within COREs at TAD boundaries led us to assess if they were themselves forming COREs, i.e. present as a cluster of binding sites at TAD boundaries. Using CREAM on the 86 and 98 ChIP-seq data from GM12878 and K562 cells, respectively, identified 41 and 59 transcription factors in either cell line forming at least 100 clusters across the genome. Comparing the distribution of transcription factor-COREs (TF-COREs) at TAD boundaries versus intra-TADs revealed more than 50% of CTCF, RAD21, SMC3, and ZNF143 TF-COREs at TAD boundaries, exemplified at the MYC and BCL6 gene loci. In contrast, less than 10% of the SP1 and GATA2 TF-COREs mapped to TAD boundaries in GM12878 and K562 cell lines, respectively. Taken together, these results suggest that clusters of CTCF and cohesin binding sites are preferentially found at TAD boundaries.

[70] FOXA1 AS A MASTER REGULATOR IN MOLECULAR APOCRINE AND LUMINAL BREAST CANCER SUBTYPES

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Transcriptome profiling in breast tumours has revealed different subtypes, but the underlying causes of subtype determination still require further investigation. Using correlation analysis in breast tumour datasets, we identified a cluster of highly-correlated luminal genes including ESR1, FOXA1 and GATA3. Importantly, expression patterns of these TFs recapitulate stratification of luminal (FOXA1^{high} ESR1/GATA3^{high}), molecular apocrine (FOXA1^{high} ESR1/GATA3^{low}) and basal-like (FOXA1^{low} ESR1/GATA3^{low}) tumours. Downregulation of FOXA1 expression in luminal cells supported a role as a positive modulator of other luminal TFs, including ESR1. In molecular apocrine SK-BR-3 cells, which are ERα-negative and GATA3^{low}, FOXA1 maintained binding to response elements present in several luminal cluster genes, although its binding to ESR1 regulatory regions was reduced and this correlated with the presence of repressive chromatin marks. Ectopic ERα expression in these cells resulted in E2-induced binding of the receptor to estrogen response elements (EREs) but

also to pre-bound FOXA1 elements, chromatin opening at some EREs with low FOXA1 binding, and activation of an E2-transcriptional response reminiscent of that observed in luminal cells. Collectively, these findings are consistent with transcriptional cooperativity between FOXA1 and ERα for expression of luminal genes and suggest that differential expression of these master regulators dictates breast tumour subtype specification.

[71] TARGETING THE METHYLOME TO BLOCK BREAST CANCER GROWTH AND METASTASIS

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Abnormal DNA methylation is a common hallmark of cancer which orchestrates many of the gene expression irregularities seen in cancer. Both hypermethylation-mediated inactivation of tumor suppressor genes as well as hypomethylation-mediated activation of prometastatic genes are observed in cancer cells which make the epigenome an attractive anti-cancer drug target. However, simultaneous targeting of both hypermethylation and hypomethylation mediated abnormalities of the cancer epigenome has never been tested in a therapeutic setting. Based on the heterogeneity of mammary tumor cells, we hypothesized that treatment with a combination of demethylating (Decitabine) and methylating agents (S-Adenosylmethionine, SAM) would collectively lead to the activation of tumor suppressor genes and suppression of pro-metastatic genes to block tumor growth and metastasis. We first investigated the effects of Decitabine and SAM alone and in combination to prevent mammary tumor growth and metastasis using the well-established and highly aggressive MDA-MB-231 xenograft model. Our data showed that both Decitabine and SAM resulted in a significant reduction of tumor volume and lung metastasis in experimental animals compared to controls. These effects were significantly more pronounced in the animals receiving the combination treatment. Gene expression analysis revealed that the combination therapy upregulates several tumor suppressor genes and also represses the expression of prometastatic genes. Immunohistochemical analysis of the primary tumors revealed that the combination treatment reduced the number of Ki-67 positive cells as well reduced the expression of angiogenesis marker CD-31. Further studies examining the effects of combined therapy on epigenome-wide methylation changes as well as any potential side effects on animal behavior and toxicity will be presented and discussed. Results from this study will provide a rationale for the initiation of clinical trials with epigenetic drugs targeting the methylome to reduce breast cancer-associated morbidity and mortality.

[72] TRANSCRIPTION PROMOTES THE INTERACTION OF THE FACILITATES CHROMATIN TRANSACTIONS (FACT) COMPLEX WITH NUCLEOSOMES IN S. CEREVISIAE

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The FACT (FAcilitates Chromatin Transactions) complex, highly conserved across eukaryotes, has long been linked with transcription. The complex is enriched on highly expressed genes, where it facilitates transcription while maintaining chromatin structure. How it is targeted to these regions is unknown. In vitro, FACT binds destabilized nucleosomes, supporting the hypothesis that FACT is targeted to transcribed chromatin through recognition of RNA polymerase-disrupted nucleosomes. In this study, we used high resolution analysis of FACT occupancy in S. cerevisiae to test this hypothesis. We demonstrate that FACT interacts with destabilized nucleosomes in vivo and its interaction with chromatin is dependent on transcription by any of the three RNA polymerases. Deep sequencing of micrococcal nuclease-resistant fragments shows that FACT-bound nucleosomes exhibit differences in micrococcal nuclease sensitivity compared to bulk chromatin, consistent with a modified nucleosome structure being the preferred ligand for this complex. Interestingly, a subset of FACT-bound nucleosomes may be “overlapping di-nucleosomes”, in which one histone octamer invades the ~147 bp territory normally occupied by the adjacent nucleosome. While the presence of altered nucleosomes associated with FACT can also be explained by the known ability of this complex to reorganize nucleosome structure, transcription inhibition alleviates this effect indicating that it is not due to FACT interaction alone. Collectively these results suggest that FACT is targeted to transcribed genes through preferential interaction with RNA polymerase-disrupted nucleosomes.

[73] NOVEL MOUSE MODEL PROVIDES INSIGHT INTO EPIGENETIC UNDERPINNINGS 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 has recently been shown to disrupt BAF complexes causing ejection of a core BAF subunit, BAF47, and altered targeting of BAF complexes. This in turn leads to both gain and loss of gene expression which drives synovial sarcomagenesis. Despite recent advancements in our understanding of the biology of synovial sarcoma, the cell of origin is still unknown and remains an obstacle to our comprehensive understanding of this disease.

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 median latency period of 16 weeks. Histologic analysis of tumours reveals characteristic cellular morphology and immunofluorescent staining confirms expression of common markers of synovial sarcoma. Single-cell RNA-seq (scRNA-seq) analyses show that gene expression in these tumours parallels transcript profiles observed in human synovial sarcoma samples and highlight genetic programs involved in sarcomagenesis.
Using this model, we can identify and isolate early neoplastic lesions prior to the development of grossly visible tumours; scRNA-seq of these lesions shows a partially-transformed phenotype and will aid in the identification of cellular programs necessary at various stages along the spectrum of synovial sarcomagenesis. Importantly, MSPCs from control animals are being used as a normal comparator to enable identification of the epigenetic differences between normal and neoplastic cells.
We have recently incorporated a conditional knockout of Baf47 into this model to further study the role of BAF47 in the development and progression of synovial sarcoma. Preliminary results indicate that bi-allelic loss of Baf47 in the context of SS18-SSX expression leads to a shortened latency period and more aggressive phenotype, providing an exciting opportunity to further evaluate the role of this subunit in the pathogenesis of synovial sarcoma.
Overall, this model identifies a putative cell of origin for synovial sarcoma and provides us with a unique opportunity to address the molecular and cellular underpinnings of synovial sarcoma.

[74] 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 in Canada, at a rate of 194 per 100,000 people. Heart disease is characterized by remodelling of the cardiac tissue in response to chronic mechanical and hormonal stress placed upon the heart. Initial remodelling increases cardiac function through hypertrophy of the heart's contractile cardiomyocytes but is ultimately maladaptive and leads to heart failure. Current treatments predominantly target the G protein-coupled receptor (GPCR) family to slow disease progression by inhibiting the hormonal signalling pathways leading to remodelling. These treatments provide benefit to a proportion of patients, but another therapeutic avenue targeting the underlying molecular changes would further improve disease prognosis.
A potential downstream target currently being explored is recruitment of the transcription regulator positive transcription elongation factor b (P-TEFb). Hormonal activation of multiple different GPCRs, such as the α_1-adrenergic receptor (α_1AR) or endothelin receptor (ETR), leads to an increase in P-TEFb activity and recruitment in cardiomyocytes. Active P-TEFb is recruited to chromatin to regulate gene expression as a constituent of two chromatin-binding complexes, either through interactions with the bromodomain and extra-terminal protein Brd4 or the Super Elongation complex (SEC). How signalling downstream of distinct GPCRs leads to activation of either complex and subsequent hypertrophic phenotype in cardiomyocytes, and how this effects their therapeutic potential, is not known.
We hypothesized signaling through distinct GPCRs elicits similar morphological and gene expression programs through distinct activation of different P-TEFb complexes. Transcriptome analysis through RNA-seq demonstrated that α_1AR or ETR activation in primary neonatal rat cardiomyocytes initiated similar P-TEFb dependent gene expression programs and hypertrophic phenotype. Although P-TEFb activity was required downstream of both receptors, the recruitment mechanism differed. The SEC was indiscriminately required, however, preventing Brd4-mediated recruitment only reduced the α_1AR-mediated gene expression and hypertrophic response. As confirmed by chromatin immunoprecipitation, recruitment of Brd4 only occurred in response to α_1AR activation. We are currently investigating a novel mechanism of Brd4 activation and recruitment through α_1-AR-mediated activation of protein kinase A (PKA). In preliminary data, PKA inhibition prevented recruitment of Brd4 in response to α_1AR activation, independently of histone acetylation changes. The activation of the Brd4/P-TEFb complex in response to selective GPCR activation has potential clinical implications as therapies targeting this complex are currently being explored for heart failure.

[75] SOMATIC MUTATIONS AND RISK-VARIANTS CONVERGE ON CIS-REGULATORY ELEMENTS TO REVEAL THE ONCOGENIC TRANSCRIPTION FACTORS IN PRIMARY PROSTATE TUMORS

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Whole-genome sequencing (WGS) of primary prostate tumors has led to the discovery of driver coding mutations but also thousands of noncoding somatic Single Nucleotide Variants (SNVs) of unknown function. Combining WGS with cis-regulatory element (CRE) annotation in primary prostate tumors, we report that somatic SNVs enrich in cistromes of a core set of oncogenic transcription factors, including the prostate master regulators AR, FOXA1, and HOXB13. This parallels enrichment of prostate cancer genetic predispositions over these transcription factors' cistromes, exemplified at the 8q24 locus harboring both risk variants and somatic SNVs in CREs upregulating MYC oncogene expression in prostate cancer cells. Using primary prostate tumors and adjacent normal tissue, we further show that the enrichment of somatic SNVs, and risk-variants, is specific to tumor as opposed to normal transcription factor cistromes. Overall, our results suggest that somatic SNVs converge with germline risk-variants on CREs of oncogenic transcription factors in primary prostate tumors.

[76] 3-DIMENSIONAL (3D) CULTURE TRIGGERS PHENOTYPIC CHANGES AND ALTERATIONS IN THE EPIGENETIC PROFILE OF CANCER CELLS.

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The cancer research and drug discovery fields rely predominantly on 2-dimensional, monolayer cell culture methods. However, these approaches do not consider key elements in tumor biology such as microenvironment, cell interactions and hypoxia. It is known that subtle changes in the microenvironment can affect the epigenome. For example, an increase in hypoxia produces acidosis, which results in epigenetic changes mainly altering histone acetylation. However, its overall impact on the epigenetic landscape has yet to be investigated. Therefore, we aimed to characterize the mechanistic, phenotypic and epigenetic differences between 2D and 3D cell cultures. To this end, A549 (adenocarcinoma) cells were seeded in a 96 well ultra-low attachment (ULA) plate promoting the spontaneous formation of spheroids. We monitored spheroid formation by live-cell analysis system. After 3D culture, from 3 days to 5 weeks, we measured a reduction of proliferation as compared to 2D culture. We also found, with flow cytometry, that cells rapidly adapted to 3D culture through reducing their size by 30% (p <0,0001) after only 72 hours. We then investigated whether 3D cell culture phenotypic changes were associated with epigenetic alterations. Strikingly, we observed a 60% upregulation in chromatin repressive marks such as H3K9me1 and H3K9me3 after only 72 hours in 3D. We also observed downregulation in histone acetyl-transferase expression KAT3a (95%) and KAT3b (60%) after 2 weeks in 3D. Furthermore, long-term tridimensional culture (5 weeks) induced an 80% upregulation in the histone deacetylase HDAC6 expression levels, which was associated with a significant downregulation of tubulin expression. In summary, we observed a cell size reduction and a reprogramming of the epigenomic signature induced by short-term and long-term 3D cell culture. Our study shows that 3D cell culture methods trigger important epigenetic changes, which should be considered while conducting cell culture assays and epigenetic studies.

[77] THE ROLE OF THE METHYLTRANSFERASE MLL4 IN MUSCLE REGENERATION

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The methyltransferase MLL4 (KMT2D) has been identified as the major histone modifier responsible for H3K4 monomethylation at active enhancers. Furthermore, it has been shown that MLL4 while dispensable for cell identity, it is essential for cellular differentiation in embryonic stem cells. However, the role of MLL4 in the regeneration of adult tissue is not as well understood. Here, we use an inducible mouse knockout to specifically ablate MLL4 in satellite cells. We show that the loss of MLL4 results in an impairment in the ability of muscle cells to regenerate. Additionally, loss of MLL4 is not necessary to maintain satellite cells in a quiescent and state, however, the activation of satellite cells is delayed. Furthermore, the ability of MLL4 knockout myoblast to proliferate is not affected while their ability to fuse and differentiate is impaired. Therefore, our results indicate that while MLL4 is not necessary to maintain cell identity, it is required for the cell fate transitions during regeneration in adult tissue.

[78] THE PREGNANT MYOMETRIUM IS EPIGENETICALLY ACTIVATED AT LABOUR-SPECIFIC GENE LOCI PRIOR TO THE ONSET OF LABOUR

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Preterm birth is the leading cause of infant death worldwide and is associated with both cognitive and physiological challenges throughout life of premature babies. Effective treatments to prevent the progression of preterm labour are lacking; this is in part due to a limited understanding of the molecular basis for changes required to initiate contractile activity in the uterine muscle (myometrium). During the transition from pregnancy to the onset of labour, myometrial smooth muscle exits a quiescent state and acquires a contractile phenotype. This physiological change is known to be associated with the expression of contractility-associated proteins, but the molecular mechanisms controlling the regulation of these genes are not well-characterised. We evaluated the epigenetic landscape of mouse myometrium during pregnancy, labour and post-partum. We targeted histone H3K27 acetylation (H3K27ac), H3K4 tri-methylation (H3K4me3), and RNA polymerase II (RNAPII) occupancy by chromatin immunoprecipitation sequencing (ChIP-seq), as well as gene expression by total RNA-sequencing (RNA-seq). Our findings reveal that, although the labour-associated contractility-driving genes are upregulated during active labor due to an increase in transcription, their promoters and putative intergenic enhancers are epigenetically activated by H3K27ac as early as three days before labour onset. Despite this early presence of H3K27ac at putative intergenic enhancers for labour-associated genes, non-coding enhancer RNAs (eRNAs) and recruitment of RNAPII to labour-associated gene bodies is only observed during active labour. Furthermore, through sequence analysis of intergenic regions producing eRNAs during pregnancy or at labour, and examination of transcription factor gene expression, we report a distinct shift in the transcription regulatory network in both mice and humans that could drive changes in gene expression at the onset of labour. Our findings offer a clearer understanding of the molecular basis of labour onset in both mice and humans and provide novel insight into mechanisms that can be targeted for the prevention of preterm birth.

[79] REPRESSION OF SOMATIC GENES BY SELECTIVE RECRUITMENT OF HDAC3 BY BLIMP1 IS ESSENTIAL FOR MOUSE PRIMORDIAL GERM CELL FATE DETERMINATION

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Primordial germ cells (PGCs) are fate-determined from pluripotent epiblasts. Signaling pathways and transcriptional regulators involved in PGC formation have been identified, but detailed molecular mechanisms of PGC fate determination remains poorly understood. We identified histone deacetylase 3 (HDAC3) as an epigenetic regulator of PGC formation by an RNAi screening. Hdac3 deficiency resulted in decreased nascent PGCs in vitro and in vivo, and somatic developmental genes were de-repressed by Hdac3 knockdown during PGC induction. We also demonstrated BLIMP1-dependent enrichment of HDAC3 and de-acetylation of H3/H4 histones in the somatic developmental genes in epiblast-like cells. In addition, the HDAC3/BLIMP1-targeted somatic gene products were enriched in PGC determinant genes; over-expression of these gene products in PGC-like cells in culture resulted in repression of PGC determinant genes. We propose that selective recruitment of HDAC3 to somatic genes by BLIMP1 and subsequent repression of these somatic genes are crucial for PGC fate determination.

[80] GFI1 TETHERS THE NUCLEOSOME-REMODELING AND HISTONE-DEACETYLASE COMPLEX TO ACTIVE CHROMATIN REGIONS IN MYELOID PROGENITORS.

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Gfi1 is known as a transcriptional repressor, which is an important regulator in myeloid differentiation and oncogenesis. Neutropenia and an accumulation of granulomonocytic progenitor cells result from Gfi1 deficiency. Here we identify the chromatin-remodelling complex, NuRD, as a significant associate of Gfi1 and its homologue Gfi1b. The NuRD complex's CHD4 and MTA2 components seem to associate with both Gfi1's N- and C-terminal and are not dependent on the presence of LSD1, a CoREST complex member and Gfi1's known potential partner. Furthermore, Gfi1 and NuRD appear to form a distinct complex from CoREST. ChIP-seq analysis reveals that Gfi1 and CHD4 notably co-localize at regulatory regions in primary myeloid progenitors. Surprisingly, they co-bind exclusively at regions of open, active chromatin enriched with H3K4 methylation and H3K27ac. Additionally, gene targets of Gfi1 and CHD4 are transcriptionally more active than genes not bound by these factors. Interestingly, the same is true of promoters bound solely by Gfi1. Loss of Gfi1 in primary myeloid progenitors leads to a loss of CHD4 enrichment at a significant proportion of target regions, but no detectable decrease in CHD4 RNA and protein levels. Gfi1 deficiency in GMPs results in only moderate gene expression alterations, with the majority of genes changing by less than two-fold. We propose that Gfi1 is present with NuRD at target regions to fine-tune gene expression in myeloid progenitors ready to repress, or not, appropriate lineage-specific genes upon myeloid differentiation.

[81] SINGLE-NUCLEUS RNA SEQUENCING SHOWS CONVERGENT EVIDENCE FROM DIFFERENT CELL TYPES FOR ALTERED SYNAPTIC PLASTICITY IN MAJOR DEPRESSIVE DISORDER.

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Major depressive disorder (MDD) is a complex illness that involves the interaction of different brain systems, pathways, and cell types. Past molecular studies of MDD relied on cellular homogenates of post-mortem brain tissue, making it impossible to determine gene expression changes within individual cells. Using single-cell transcriptomics, we examined almost 80,000 nuclei from the dorsolateral prefrontal cortex of individuals with MDD and healthy controls. Our analyses identified 26 distinct cellular clusters, and over 60% of these showed transcriptional differences between groups. Specifically, 96 genes were differentially expressed, the majority of which were downregulated. Convergent evidence from our analyses, including gene expression, differential correlation, and gene ontology implicated dysregulation of synaptic plasticity in the etiopathogenesis of MDD. Our results show that this high-resolution approach can reveal previously undetectable changes in specific cell types in the context of complex phenotypes and heterogeneous tissues.

[82] CIRCADIAN DNA MODIFICATION IS INVOLVED IN AGING AND COMPLEX DISEASE

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Epigenetic factors are involved in the circadian machinery; however, despite extensive efforts, detection and characterization of circadian DNA (cytosine) modification at a nucleotide level has been elusive. We performed a comprehensive mapping of oscillating modified cytosines (osc-modC) in mouse tissues and detected that osc-modCs belong to the category of cytosines exhibiting the high degree of inter-

individual variation, which has traditionally been treated as stochastic (Oh et al. Nature Comm 2018). In addition, osc-modCs showed several links to the circadian transcriptome and, importantly, were associated with the aging epigenome. Osc-modCs preceded age-dependent DNA modification changes, and osc-modC amplitude strongly correlated with the magnitude of the aging effect. The linking of two temporal dimensions (dynamic circadian cycles and more stable molecular aging) provides a new interpretation of aging and its proximal causes.
Since aging is closely related to human disease, we asked if there were any associations between osc-modCs and human diseases. For this, we performed an analysis of epigenome-wide association studies of four human diseases: chronic lymphocytic leukemia, schizophrenia, obesity (BMI), and type II diabetes. We detected that differentially modified cytosines in disease were significantly associated with human neutrophil osc-modCs (odds ratio ranged from 1.2 to 8.1; with p values ranging from 0.0032 to 2.8E- 47), which indicates that circadian epigenomic dysregulation is involved in etio-pathogenesis of disease.
In summary, circadian DNA modifications may provide a new perspective on several fundamental questions in biomedical research. In the broad sense, circadian rhythmicity, one of key adaptations of life on Earth, seems to also be mediating an organism's demise.

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[83] DISTINCT REGULATORY ROLES OF KDM6 FAMILY HISTONE DEMETHYLASES UTX AND JMJD3 DURING MUSCLE REGENERATION

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Histone methylation dynamics modulated by methyltransferases and demethylases maintain a decisive role in cell fate transition during development. To ensure the proper spatial and temporal activation of lineage-determination genes, spurious transcription is impeded by repressive epigenetic marks of histone H3 lysine 27 trimethylation (H3K27me3). To permit expression of lineage-specific genes, H3K27me3 marks are removed by the KDM6 demethylase family proteins UTX (KDM6A) and JMJD3 (KDM6B). UTX and JMJD3 proteins both harbor a highly homologous Jmjc domain that facilitates H3K27me3 demethylation but diverge in their N-terminal domains which are predicted to mediate protein-protein interactions. As muscle stem cells (here after called satellite cells) express both UTX and JMJD3, we set out to determine the role for these distinct H3K27-demthylases in muscle regeneration. Mice were generated with a satellite cell-specific inducible knockout (KO) for either UTX or JMJD3. Using a cardiotoxin-induced injury model, we find that JMJD3 and UTX play non-redundant roles that are critical for satellite cell-mediated regeneration. Characterization of $UTX^{-/-}$ and $JMJD3^{-/-}$ satellite cells in culture showed that JMJD3 is specifically required for the proliferation of myoblast population. In contrast, UTX was dispensable for proliferation but was critically required for differentiation to form myotubes. ChIP and RNA-Seq analysis has provided important insight into the divergent roles of UTX and JMJD3 in muscle regeneration. This work represents the first demonstration that UTX and JMJD3 can play non-overlapping role in regulating the expansion and differentiation of stem cell populations to mediate regeneration.

[84] ROLE OF MACROH2A2 IN THE GLIOBLASTOMA STEM CELL EPIGENOME

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Glioblastoma is the most common primary malignant brain tumour in adults and remains uniformly lethal despite aggressive treatment protocols. These tumours have a hierarchical organisation, with a subpopulation of glioblastoma stem cells (GSCs) that drive tumour recurrence and drug resistance. One important factor that divides GSCs from non-GSCs is their epigenome, one element of which is the histone variant code.
MacroH2A2 is a histone variant that can stratify glioblastoma patients, with higher levels of this histone variant associated with better patient prognosis. Knockdown of macroH2A2 in GSCs is associated with increased self-renewal and an increased expression of stemness genes such as SOX2, SOX5 and ASCL1 by RNA-seq. Increasing levels of macroH2A2 are also seen with GSC differentiation. Knockdown of macroH2A2 also leads to repositioning of nucleosomes around the transcriptional start site of certain genes by ATAC-seq, suggesting a role for this histone in fine-tuning gene expression.
As elevation of macroH2A2 is associated with reduced self-renewal, we performed an in vitro high content screen with an epigenetic drug library at sublethal doses to identify compounds that upregulate macroH2A2 within GSC cultures. We developed a semi-automated analysis pipeline to stratify cells based on viability and macroH2A2 immunofluorescence. Menin inhibitors were found to increase the proportion of macroH2A2-high cells, in keeping with their previous role in promoting differentiation.
Our preliminary results suggest that macroH2A2 is a novel biomarker for glioblastoma and that macroH2A2 loss is a marker of GSC stemness and a poor prognostic marker in glioblastoma. This work identifies loss of macroH2A2 as a feature of GSCs and provides a framework for therapeutic modulation of this histone variant in GSCs.

[85] DNA METHYLATION MARKS IN NUCLEAR AND MITOCHONDRIAL DNA

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Epigenetic mechanisms regulate all biological processes from conception to death, including genome reprogramming during

early embryogenesis and gametogenesis, cell differentiation, and maintenance of a committed lineage. DNA methylation is the most extensively studied epigenetic modification with 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) being key epigenetic players. Interestingly, mitochondrial DNA (mt DNA) is also believed to be modified by both 5-mC and 5-hmC; nevertheless, the presence and function of such epigenetic modifications are still highly debated. Moreover, mt DNA cellular content differs across tissue types depending on several factors particularly on the energy demand of the cells which influence mt DNA replication and transcription rate. However, the ratio of mitochondrial DNA relative to the nuclear genome in leukocytes (CD4+ and CD8+ T cells) is not well studied. Peripheral blood mononuclear cells (PBMCs) are frequently used in DNA methylation studies and considered an informative study model with CD4+ and CD8+ T cells being reported as "most distinctly poised for rapid methylome response to physiological stress and disease". So, our objective is to investigate the differentially methylated and hydroxymethylated regions in nuclear and mitochondrial DNA of CD4+ and CD8+ T cells and the relative ratio of mitochondrial to nuclear genome in these cells to roughly estimate whether the changes in methylation levels could possibly be contributed to changes in their mitochondrial DNA cellular content. In this study, whole blood samples were collected from healthy donors and PBMCs were sorted into CD4+ T and CD8+ T cells for analyses of 5-mC and 5-hmC in total cellular DNA. MethylMiner protocol and hydroxymethylated DNA immunoprecipitation were used to enrich for methylated and hydroxymethylated DNA fragments. The samples are being sequenced and bioinformatic analysis for the nuclear and mitochondrial methylome will be done. Significant variations in the DNA methylation levels between those cells are predicted to be present and might likely be influenced by their relative mitochondrial/ nuclear DNA ratio. Overall, this will broaden our understanding of the cell-type-specific epigenetic events in normal physiological state which in turn will impact the future epigenetic analysis of the disease state.

[86] FOLATE DOSE AND FORM DURING PREGNANCY DIFFERENTIALLY AFFECT METHYLATION POTENTIAL AT BIRTH AND LATER LIFE BODY WEIGHT-GAIN AND ENERGY EXPENDITURE IN WISTAR RAT MOTHERS AND FEMALE OFFSPRING

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The transition into motherhood is characterized by distinct physiological changes and diet during pregnancy is a determinant of her long-term metabolic consequences. Folic acid (FA, synthetic folate) is widely recommended for neural tube defect prevention but intakes by pregnant women in North America exceed recommendations by 2.5-7-fold. Folates function as primary constituents of the 1-carbon cycle, mediating DNA-methylation dependent gene expression. However, high intakes may lead to perturbations modulating the availability of the methyl donor S-adenosylmethionine (SAM) and activity of DNA methyltransferases (DNMTs). Recently, 5-methyltetrahydrofolate (5MTHF), the bioactive form of folate, has gained popularity as a supplement. However, a comparison of these folate forms during pregnancy on long-term health outcomes have not been reported. This is the first study to investigate whether the dose (low vs high) and form (FA vs 5MTHF) of folate during pregnancy differentially affects energy balance pathways in the Wistar rat mother and female offspring through DNA methylation-dependent regulation of gene expression.
Pregnant Wistar rats (n=22/group) were fed an AIN-93G diet with either recommended FA (1X, control, 2mg/kg diet), 5X-FA or equimolar levels of 5MTHF. Dams were fed the control diet during lactation and then dams and female pups were fed a high fat (45% fat) diet post-weaning for 19 weeks. Dependent measures include: body weight-gain, food intake, 24hr energy expenditure, fasting plasma energy balance hormones, insulin resistance, and levels of SAM, S-adenosylhomocysteine (SAH) and DNMT activity at birth and post-weaning in dams and female offspring (mothers-to-be).

Our results show a lasting and differential effect of the folate diets consumed during pregnancy on Wistar rat mothers and female offspring. Dams fed 5X-MTHF diets had higher DNMT activity at birth and their female pups had higher SAM:SAH levels indicative of higher methylation potential. Dams fed 5X-FA maternal diets tended to have higher plasma leptin ($p=0.06$) at birth and had lower insulin resistance ($p<0.05$) at 1-week post-weaning compared 5X-MTHF dams, highlighting the unique effects of the diets to enable re-set of maternal metabolism. In both dams and female offspring, 5X-FA maternal diets led to delayed post-weaning weight gain (~25%, $p<0.001$) and lower 24hr energy expenditure ($p<0.05$) without affecting cumulative food intake. Plasma hormones and insulin resistance were similar across all diet groups once fed the high-fat diet.
In conclusion, folate dose and form during pregnancy differentially affects DNA methylation potential at birth and the early- and later post-partum phenotype of the Wistar rat mother and female offspring. More research on DNA methylation-dependent mechanisms regulating these later life outcomes are underway.

[87] TFAP2C REGULATES TRANSCRIPTION IN HUMAN NAÏVE PLURIPOTENCY BY OPENING ENHANCERS.

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Naive and primed pluripotent human embryonic stem cells (hESCs) bear transcriptional similarity to pre- and post-implantation epiblast and thus constitute a developmental model for understanding the pluripotent stages in human embryo development. To identify new transcription factors that differentially regulate the unique pluripotent stages, we mapped open chromatin using ATAC-seq and found enrichment of the activator protein-2 (AP2) transcription factor binding motif at naive-specific open chromatin. We determined that the AP2 family member TFAP2C is specifically upregulated in naïve hESCs and widespread at naïve enhancers. Using TFAP2C-deficient and inducible lines, we determined that TFAP2C functions to promote naïve pluripotency by facilitating the opening of enhancers of genes expressed during pre-implantation development, including the key mammalian pluripotency factor POU5F1 (OCT4). These naïve-specific enhancers also show openness in human pre-implantation embryos, supporting their functionality in vivo. Taken together, our data indicates that TFAP2C establishes and maintains naive human pluripotency and directly regulates OCT4. Our data also support a role for TFAP2C in both opening chromatin and maintaining the activity of enhancers.

[88] ROLE OF THE ATRX CHROMATIN REMODELING PROTEIN IN ASTROCYTES

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Astrocytes are glial cells of the central nervous system (CNS) that regulate neurotransmitter and nutrient metabolism as well as synapse formation, maturation and elimination. Astrocyte participation in neuronal function is a carefully regulated process that depends on the expression of specific genes. Defective astrocytes can alter neuronal function and contribute to disease, as has been reported in monogenic neurodevelopmental disorders like Rett and Fragile X syndrome. Alpha Thalassemia X-linked intellectual disability is a neurodevelopmental disorder caused by mutations in the ATRX gene. A model for Cre/loxP tamoxifen-inducible Atrx deletion in astrocytes (Atrx f/f;dTom;GlastCreER) was generated to directly investigate the effects of ATRX loss of function on astrocyte transcriptomic and epigenomic profiles, as well as potential deleterious effects on neuronal morphology and neurobehaviours.
The outcome of Atrx deletion in astrocytes on working memory (Y maze), recognition memory (Novel Object Recognition) and spatial memory (Morris Water Maze) was evaluated in 3-6-month-old mice. No difference was detected between astrocyte Atrx KO (ATRX KO) and control animals for working or short-term recognition and spatial memory. However, preliminary results suggest alterations in long-term recognition and spatial memory in ATRX KO mice. To evaluate the effect of Atrx deletion in astrocytes on the transcriptome and epigenome, GFP-positive astrocytic nuclei were isolated from the forebrain of Atrx f/Y; Sun1-GFP; GlastCreER and control mice using the INTACT protocol and processed for ATAC- seq and RNA-seq to assess chromatin accessibility and identify genes and pathways regulated by ATRX in astrocytes. The INTACT model and protocol have been validated and optimized to obtain astrocytic nuclei from ATRX KO and control forebrain. Our study demonstrates that the loss of the ATRX epigenetic factor specifically in the astrocytes of mice leads to spatial and recognition memory impairments linked to specific epigenetic alterations.

[89] UNDERSTANDING THE ROLE OF PATERNAL OBESITY IN THE TRANSMISSION OF METABOLIC SYNDROME ACROSS GENERATIONS

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Although there is increasing evidence that paternal preconception environment can affect offspring health, the nongenetic molecular mechanisms underlying complex disease inheritance are still elusive, and the role of histone methylation in this phenomena is unknown. The Kimmins lab has previously established that sperm histone methylation is implicated in transgenerational epigenetic inheritance, using a transgenic mouse model with a compromised sperm epigenome which gave rise to abnormal offspring with reduced survivability.
We sought to investigate whether high-fat diet associated with elevated BMI could compromise the methyl donor pool and thereby alter the sperm epigenome which could explain metabolic disease transmission across generations.
We hypothesized that descendants derived from a paternal line with an altered sperm epigenome will be more sensitized to the detrimental effects of an environmental challenge in the form of a high-fat diet.
To test this hypothesis, wildtype (n=17 and 25), transgenic (n=15 and 24) and wildtype littermates (n=13 and 23) males were fed for 12 weeks a low- or a high-fat diet (10% and 60% kcal fat respectively). The F1 and F2 generations were generated by breeding males to 6-weeks-old wildtype females. Females for breeding and F1 and F2 animals were fed a regular chow diet. All animals were tested for metabolic function at 4 months of age by intraperitoneal glucose and insulin tolerance tests, as well as fasting blood glucose and serum insulin levels.
Males on a high-fat diet became obese, glucose intolerant, insulin insensitive and hyperglycemic. Interestingly, sex-specific effects were noted where male descendants of high-fat fed fathers or grandfathers showed altered metabolic responses. In contrast, none of the female offspring and grand offspring had altered metabolic function resulting from their ancestors' diet nor genotype. Additionally, it was observed that males sired by non-transgenic and transgenic obese males had exacerbated metabolic responses compared to wildtype animals. We are currently assessing the sperm epigenome of males fed a high-fat diet in order to detect epigenomic signatures in terms of histone methylation associated with diet and offspring metabolic functions.

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[90] RECAP REVEALS THE TRUE STATISTICAL SIGNIFICANCE OF CHIP-SEQ PEAK CALLS

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ChIP-seq is used extensively to identify regions of epigenetic modifications to the genome. The fundamental bioinformatics problem is to take ChIP-seq read data and data representing some kind of control and determine genomic regions that are enriched in the ChIP-seq versus the control, also called "peak calling." While many programs have been designed to solve this task, nearly all fall into the statistical trap of using the data twice--once to determine candidate enriched regions, and a second time to assess enrichment by methods of classical statistical hypothesis testing. This double use of the data has the potential to invalidate the statistical significance assigned to enriched regions, or peaks, and as a consequence, to invalidate false discovery rate estimates. Thus, the true significance or reliability of peak calls remains unknown. Using simulated and real ChIP-seq data sets, we show that three well-known peak callers, MACS, SICER and diffReps, output optimistically biased p-values, and therefore optimistic false discovery rate estimates—in some cases, many orders of magnitude too optimistic. We propose a wrapper algorithm, RECAP, that uses resampling of ChIP-seq and control data to estimate and correct for biases built into peak calling algorithms. P-values recalibrated by RECAP are approximately uniformly distributed when applied to null hypothesis data—that is, data where ChIP-seq and control come from the same genomic distributions. When applied to non-null data, RECAP p-values give a better estimate of the true statistical significance of candidate peaks. In turn, this leads to better false discovery rate estimates, which correlate better with empirical reproducibility. RECAP is a powerful new tool for assessing the true statistical significance of ChIP-seq peak calls.

[91] THE SS18-SSX ONCOPROTEIN IS DIRECTED BY DNA METHYLATION STATE TO EVICT POLYCOMB IN PRIMARY SYNOVIAL SARCOMAS.

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Background: Synovial sarcoma is characterized by a balanced chromosomal translocation t(X,18; p11,q11), resulting in the production of a fusion oncoprotein, SS18-SSX. Recent models based on cell line experiments have proposed that the oncoprotein takes advantage of other complexes, including KDM2B-PRC1.1, a noncanonical polycomb repressive complex, and mammalian SWI/SNF (BAF), a chromatin remodelling complex, to drive this cancer. Specifically, KDM2B binds unmethylated CpG islands (CGIs), recruiting SS18-SSX and BAF, and ultimately activates genes that would be otherwise repressed.
Methods: We have profiled 5 synovial sarcoma primary tumors using a Post-Bisulfite Adapter Ligation (PBAL) protocol for DNA methylation, RNA-seq for transcriptome data, and Chromatin Immunoprecipitation sequencing (ChIP-seq) for 6 core histone modifications (H3K4me3, H3K4me1, H3K9me3, H3K27me3, H3K27ac, H3K36me3). RNA-seq and ChIP-seq were similarly performed on 2 synovial sarcoma mouse model tumors. Publicly-available RNA-seq and ChIP-seq data (histone modifications, KDM2B and SS18-SSX) were obtained for control, SSX and KDM2B knockdowns in the HSSYII and Aska synovial sarcoma cell lines.
Results: Using ChIP-seq datasets from the HSSYII cell line, SS18-SSX and KDM2B showed co-occupancy at their binding sites. These binding sites demonstrated eviction of H3K27me3 in HSSYII control compared to KDM2B knockdowns. However, such a relationship did not hold true in the primary tumors, suggesting SS18-SSX and KDM2B binding sites may vary amongst individual synovial sarcomas. Moreover, previously established synovial sarcoma gene signatures from cell lines were not consistent in the primary tumors. Greater variability was also seen in CGI methylation of primary synovial sarcomas compared to neuroprogenitor cells. KDM2B binding was associated with hypomethylated CGIs in primary synovial sarcomas, and this may define unique SS18-SSX recruitment in each individual tumor. A subset of genes significantly downregulated following HSSYII KDM2B knockdown and targets of SS18-SSX binding showed increased H3K27me3 signal at their promoters in KDM2B and SSX knockdowns compared to the primary synovial sarcomas. Genes associated with super-enhancers, regions significantly enriched in H3K27ac density, that were most upregulated possessed promoters that were active in both the human primary and mouse synovial sarcoma tumors, but bivalently marked (H3K4me3 and H3K27me3) in their respective non-malignant comparisons, SSX knockdown of Aska cell line and mouse muscle.
Conclusion: Using primary human and mouse model synovial sarcomas, we integrated models from cell lines that suggest that KDM2B binds hypomethylated CpG islands, and that these regions differ between individual tumors based on their unique DNA methylation states. KDM2B may then recruit SS18-SSX and the BAF complex to evict polycomb and thus activate genes, including bivalently marked genes, which drive tumorigenesis.

[92] UNIQUE REQUIREMENTS FOR THE H3K4 METHYLTRANSFERASES TRX IN DROSOPHILA LONG-TERM MEMORY

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Memory is the ability of an organism to retain information after a period of learning. Memory can be divided into two phases; short-term memory (STM) is transient, while long-term memory (LTM) is stable and can persist for years. Functionally, LTM requires de novo gene transcription and protein synthesis, whereas STM does not. The goal of our study is to further understand how

epigenetic regulators contribute to the regulation of gene transcription required for LTM. Previously, H3K4 methyltransferases have been implicated broadly in memory, however, the specific writers of this chromatin mark and the relevant target genes involved in LTM are not known. To address this, we screened all H3K4 methyltransferases (Set1, trr, and trx) in Drosophila melanogaster, for memory phenotypes using targeted RNAi knockdown (KD) restricted to the Drosophila mushroom body (MB), the fly brain's learning and memory centre. While MB-specific KD of trr and Set1 caused STM deficits, trx KD only effected LTM, leaving STM intact. This suggested trx as a strong candidate for regulating LTM specific processes over other H3K4 methyltransferases. We next demonstrated that the trx KD memory deficits were the result of adult-specific processes via temporal KD of trx during development or the adult stage, with only the adult KD presenting dysfunctional LTM. Therefore, trx must regulate LTM through regulation of gene expression in adult animals, and not through its role in development.

To identify target genes and pathways misregulated in trx KD MBs we used cell type specific RNA-sequencing from MB nuclei. We found that trx is required for expression of genes that are known to be specifically enriched in the MB. This suggests a role for trx in maintaining cell type specificity in the MB. Lactate dehydrogenase (Ldh), one of the most highly enriched MB-specific genes, was strongly down regulated in our trx KD transcriptome analysis. Due to the high energy demand of MB neurons after learning we suspect Ldh plays an important role in reaching these energy demands during memory formation, and our data suggests that trx is required to epigenetically maintain MB-specific Ldh expression.

This study has identified the H3K4 methyltransferase trx as a specific regulator of LTM in Drosophila and has revealed candidate target genes, including Ldh, that may be regulated in memory formation downstream of trx. Interestingly, trx orthologues have previously been associated with human cognitive disorders and these findings are important for elucidating potential paths to different clinical presentations.

[93] CANDIDATE NON-CODING DRIVER MUTATIONS IN SUPER-ENHANCERS AND LONG-RANGE CHROMATIN INTERACTION NETWORKS ACROSS >1,800 WHOLE CANCER GENOMES

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A catalogue of mutations that drive tumorigenesis and progression is essential to understanding tumor biology and developing therapies. Cancer driver mutations are positively selected in somatic genomes and thus appear statistically over-represented in cancer genes across large patient cohorts. Protein-coding driver mutations have been well-characterized by large exome-sequencing studies; however many tumors have no mutations in protein-coding drivers and few non-coding drivers besides the TERT promoter are known. To fill this gap, we analyzed 150,000 cis-regulatory regions in 1,844 whole cancer genomes from the largest uniformly processed whole genome sequencing dataset compiled by the ICGC-TCGA Pan-cancer Analysis of Whole Genomes (PCAWG) project. We developed a new cancer driver discovery method, ActiveDriverWGS, validated it on protein-coding genome and found 47 genes of which >90% are known drivers. We then applied the model to cis-regulatory regions from ENCODE and found 41 frequently mutated regulatory elements (FMREs) enriched in non-coding SNVs and indels. FMREs were characterized by aging-associated mutation signatures and frequent structural variants, highlighting their importance in cancer. FMREs were enriched in super-enhancers and long-range chromatin interactions, suggesting that mutations in these regions drive cancer by altering transcriptional programs of distally located genes. The chromatin interaction network of FMREs, chromatin loops and target genes revealed a subset of FMREs where mutations associated with differential gene expression of known and novel cancer genes, activation of hallmark cancer pathways and altered enhancer marks. Thus, distal genomic regions may include additional, infrequently mutated drivers that act on target genes via long-range interactions mediated by chromatin loops. Our study is an important step towards finding such frequently mutated regulatory regions and deciphering the somatic mutation landscape and disease mechanisms of the non-coding genome.

[94] MAINTENANCE OF STEM CELL CHARACTER IN TUMOUR INITIATING CELLS BY THE ING5 PROTEIN

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The ING family of epigenetic regulators target histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes to the H3K4me3 chromatin mark to alter histone acetylation and subsequently, gene expression. Targeting is mediated by interaction of the conserved plant homeodomain of the ING proteins with H3K4Me3, with ING proteins interacting with H3K4Me3 ten time more avidly than with H3K4Me2, which is ten-fold stronger that their interaction with H3K4Me. ING5 is a stoichiometric member of both the HBO1 and Moz/Morf HAT complexes that target histone H4 and histone H3, respectively, and ING proteins have also been reported to affect DNA methylation through their interactions with the growth arrest and DNA damage protein Gadd45a and associated proteins, suggesting that they can affect at least two major epigenetic mechanisms. Using an unbiased screen, ING5 was one of five proteins directly implicated in the maintenance of "stemness" in epidermal stem cells and we find that ING5 is highly expressed in different embryonic stem cell populations. ING5 is also expressed at high levels in stem cell-like brain tumour initiating cells (BTICs) compared to differentiated cells. BTICs are cells isolated from glioblastoma patients, that have the ability to initiate tumours when introduced ectopically in animal models and are thought to be the cancer cell reservoir responsible for brain tumour recurrence after treatment. Ectopic expression of ING5 promotes BTIC self-renewal, prevents lineage differentiation and increases the stem

cell pool in the BTIC population. ING5 knockdown promotes differentiation and inhibits the sphere formation that is one measure of "stemness" in BTIC cells. ING5 also increases expression of the OCT4, OLIG2 and Nestin stem cell markers and enhances mitogenic signaling in the absence of growth factors to sustain self-renewal of BTICs. Microarray data suggest that ING5 exerts these effects primarily through two major pathways; by inducing expression of calcium channels and by activating the follicle-stimulating hormone pathway. We show that both FSH and Ca^{2+}-responsive pathways impinge on BTIC stem cell character, suggesting that these pathways may serve as targets to help eliminate cancer stem cell reservoirs.

[95] ZYGOTIC DNA METHYLATION ACQUISITION IMMEDIATELY FOLLOWING FERTILIZATION IS MEDIATED BY DNMT3A AND MAY REGULATE SPERM-SPECIFIC GENES.

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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 to form the totipotent zygote. To track parent-specific epigenome remodeling in the developing zygote, we developed MEA, a computational pipeline for integrated allele-specific profiling of NGS-based DNAme, histone PTMs and transcription datasets (Richard Albert et al. 2018, BMC Genomics). Utilizing publicly available whole genome bisulphite sequencing (WGBS) in gametes and F1 hybrid 2-cell mouse zygotes to measure DNAme dynamics with single nucleotide resolution, we observe a genome-wide decrease of 43% and 7% DNAme on the paternal and maternal genomes, respectively, consistent with previous immunofluorescence data. Surprisingly, against a backdrop of global paternal genome demethylation, we uncover robust DNAme gain at 3% of all hypomethylated spermatozoa loci, including at the CpG-rich transcription start sites (TSSs) of annotated genes. A subset of these TSSs maintain such DNAme at the blastocyst stage, suggesting that epigenetic silencing of such genes may be required for normal preimplantation development. To determine whether DNMT3A, the de novo DNA MethylTransferase highly expressed in oocytes, is responsible for zygotic DNA methylation acquisition, we performed WGBS on wild type and maternal KO 2-cell mouse zygotes. Indeed, de novo DNAme at these antithetical CpG-rich TSSs was ablated in the absence of maternal DNMT3A. Using this maternal Dnmt3a KO model, we will next test (via RNAseq) whether de novo DNAme does indeed silence developmentally important genes in the 2-cell zygote, which may explain the models observed embryonic lethal phenotype. These results demonstrate the value of enhancing the level of resolution in NGS dataset analyses and reflect the potential for future discoveries using MEA.

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[96] INVESTIGATING THE ROLE OF NEGATIVE ELONGATION FACTOR IN MUSCLE REGENERATION

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Our current model of myogenesis specifies that myogenic regulatory factors (Pax7, Myf5, MyoD, and Myogenin) control the dynamic epigenetic landscape of muscle progenitor cells during regeneration. Although these changes render DNA more accessible, the specific mechanisms regulating gene expression during myogenesis are not yet fully understood. Promoter-proximal transcriptional pausing is a fitting model that explains myogenic gene regulation, given its ability to restrain an engaged RNA Polymerase II (RNA Pol II) at the promoter proximal region through association with factors such as the Negative Elongation Factor (NELF). This maintains engaged RNA Pol II stalled until extrinsic signaling alleviates the NELF-mediated pausing and allows rapid gene expression.
We hypothesize that NELF-mediated promoter proximal pausing controls myogenic gene expression to allow effective cell fate transitions of myogenic precursors.
Using a satellite cell-specific knockout mouse, we find that loss of NELF-B causes a delay in injury-induced muscle regeneration resulting in smaller muscle fibers. This phenotype is further exacerbated after muscle reinjury. RNA-sequencing of satellite cells 5-days after injury confirms a role for NELF in regulating expression of genes involved in cell cycle. These results suggest that NELF is required for efficient activation of satellite cells after muscle injury. Ongoing studies are targeted at identifying specific genes regulated by NELF-B that mediate satellite cell activation after injury.
A complete understanding of molecular processes that allow NELF to regulate transcription during myogenesis will help us better understand how myogenic precursors change cell fates, which will help develop novel therapeutic approaches for muscle degenerative disease.

[97] SYNTHETIC GLUCOCORTICOIDS DURING PREGNANCY AND EPIGENETIC OUTCOMES IN THE BRAIN AND BLOOD OF OFFSPRING IN A GUINEA PIG MODEL

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Synthetic glucocorticoids (sGC) are administered to pregnant women at risk of preterm delivery, approximately 10% of all pregnancies. Studies in animal models have shown that offspring exposed to elevated glucocorticoids, either by administration of sGC or endogenous glucocorticoids as a result of maternal stress are at increased risk of developing behavioral, endocrine,

and metabolic dysregulation. DNA methylation has been proposed to play a critical role in long-lasting programming of gene regulation that underlie behavioural and physiological phenotypes in offspring. Peripheral tissues such as blood are an accessible source of DNA for epigenetic analyses in humans. Here, we examined the hypothesis that prenatal sGC administration alters DNA methylation signatures in offspring hippocampus and whole blood. We compared these signatures across the two tissue types to assess epigenetic biomarkers potentially predictive of common underlying molecular pathways affected by sGC exposure. Pregnant guinea pigs were treated with sGC in late gestation and DNA methylation in approximately 3.7 million CpG sites was analyzed using reduced representation bisulfite sequencing in the hippocampus and whole blood from juvenile female offspring. Results indicate that there are tissue-specific methylation signatures of prenatal sGC exposure. Furthermore, among differentially methylated genes, 179 genes are modified in both brain and blood. Gene pathway analyses of these genes show that prenatal sGC exposure alters methylation of gene clusters involved in brain development and cardiovascular functions. These data may indicate a coordinated epigenetic programming in response to alterations in glucocorticoid signaling.

[98] DECREASE IN DNA METHYLATION 1 (DDM1) CONFINES THE R-LOOPS TO MAINTAIN THE PERICENTROMERIC HETEROCHROMATIN

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Processes of heterochromatin packing are essential for gene silencing, chromosome organization and genome integrity. Surprisingly, recent genome-wide identification of R-loops in Arabidopsis has revealed that these three-stranded chromatin structures are particularly enriched in pericentromeric heterochromatin regions. However, it is still unknown if R-loops have function in pericentromeric heterochromatin formation. Through a quantitative genome-wide profiling screen to identify the regulators of pericentromeric R-loops, we identified DDM1 (Decrease in DNA methylation 1) as a major restrictor of pericentromeric R-loops. Loss-of-function ddm1 mutants cause R-loops accumulation associated with decrease in heterochromatic modifications H3K9me2, H3K27me1 and DNA methylation. In vivo and in vitro evidence shows that DDM1 binds to R-loops and restricts their accumulation through its N-terminal DEXDc helicase domain. Destabilization of R-loops by RNase H1 overexpression in ddm1 mutants facilitates the re-deposition of heterochromatic marks such as H3K9me2, H3K27me1, and CHH and CHG DNA methylation, leading to heterochromatin reformation and ultimately gene silencing. Our work unravels for the first time the importance of R-loops in restricting pericentromeric heterochromatin formation in Arabidopsis, and as well as a role of DDM1 in R-loop homeostasis mediated heterochromatin formation and transcriptional gene silencing.

[99] ROLE OF NON-CANONICAL H3K36ME3 IN ESTABLISHING SPERMATOGENIC DNA METHYLATION LANDSCAPE

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Male infertility is a significant problem for human reproduction affecting 1 in 20 men. Disruption of epigenetic regulations in the male germline is implicated in a number of disorders such as oligozoospermia. Also, the incidence of congenital diseases involving DNA methylation errors in the offspring has been increased by assisted reproductive technology (ART). Thus, establishing faithful spermatogenic epigenome is a key to generate functional gametes.
Germ cells undergo extensive DNA methylation re-organization involving erasure of DNA methylation in the early stage of germ development followed by establishment of DNA methylation in a sex-dependent manner. In the mouse male germline, global de novo DNA methylation commences before embryonic day 15.5 (E15.5) and requires de novo DNA methyltransferases DNMT3A, DNMT3B and their co-factor DNMT3L. However, the molecular basis directing these enzymes to the specific genomic regions remains unclear. Utilizing publicly available whole-genome bisulfite sequencing in germ cells (GCs) at stages before (E13.5) and after (E16.5) the onset of de novo DNA methylation, we identified genomic regions with resistance to de novo DNA methylation. Intersecting with histone modification states at E16.5 male GCs has revealed that H3K9me3 is enriched at several of these resistant regions. Unexpectedly, H3K36me3, a mark associated with high DNA methylation at actively transcribed genic regions in other cell types, is not enriched at transcribed genic regions but at H3K9me3 marked regions in E16.5 male GCs. Furthermore, these non-canonical H3K36me3 regions have less DNA methylation even in mature sperm compared to other genomic regions, suggesting antagonistic relationship between DNA methylation and H3K36me3 in the male GC development. Ongoing genome-wide analysis on the mutant male mice lacking each of the responsible enzymes/factors of H3K9me3 or H3K36me3 will provide the mechanistic insights into the relationship between DNA methylation and histone modifications and the roles of non-canonical H3K36me3 in the male germline.

[100] IDENTIFICATION OF HISTONE H3.1 AND H3.3 BINDING PARTNERS REVEALS NOVEL BINDING PARTNERS AND ASSOCIATIONS WITH HISTONE CHAPERONES

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Histones form the core of the nucleosome around which DNA is wound and condensed into chromatin. Histone H3 exists in canonical, replication-coupled (H3.1), and variant, replication-independent (H3.3), forms. Histones are subject to many post-translational modifications that regulate chromatin function and

are bound by enzymes that deposit and remove these modifications, as well as machinery associated, among other things, with chromatin structure, transcription, and DNA replication. Histone interactors have previously been identified by affinity purification coupled to mass spectrometry (AP-MS), however histone extraction from chromatin requires harsh conditions such as high salt, which will disrupt many interactions. Previously characterised interactions include histone chaperones: CAF-1 has been reported to selectively deposit H3.1 into chromatin during DNA replication, while HIRA specifically deposits H3.3 into regions of active chromatin. Here we used proximity biotinylation (BioID) to map the H3.1 and H3.3 interactomes in an unbiased manner, identifying 608 high-confidence interactors involved in 80 protein complexes. Over half (351) were novel interactors not previously detected by AP-MS. Many had chromatin-, DNA replication- or transcription-related roles, and might be anticipated to have a transient interaction with histones that would be difficult to capture by traditional methods. Unexpectedly we also identified many proteins with roles in processes such as mitosis (cohesin, MIS12/18, CPC complexes), nucleolar functions (PeBoW complex [involved in 60S ribosome maturation], RNA exosome) and the DNA damage response. Strikingly, while we confirmed similar binding to NASP and ASF1, which do not discriminate between H3.1 and H3.3, and H3.3-specific binding of the HIRA complex, the interaction with CAF-1 was identical between H3.1 and H3.3, suggesting that CAF-1 binds both histone variants in vivo. This conclusion is supported by proximity ligation assays. Among other differential interaction partners, H3K9 methylases and demethylases were enriched with H3.3, suggesting higher H3K9me3 turnover in H3.3-containing genomic regions. In contrast, H3.1-enriched interactors were associated with mitotic functions. Overall, our results indicate that BioID provides a useful tool for profiling interactomes of proteins located in difficult to access cell compartments such as chromatin, as well as capturing transient or infrequent interactions.

[101] THE COMPETITION FOR COFACTORS DICTATES THE CELLULAR RESPONSE TO ENVIRONMENTAL PERTURBATIONS

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Fine tuning of the transcriptional program requires the competing action of multiple protein complexes in a well-organized environment. Genome folding creates proximity between genes, leading to accumulation of regulatory factors and formation of local microenvironments. This high level of organisation is important for different aspects of gene regulation, but questions remain regarding the dynamic and communication between genes sharing the same physical environment. Here we show that independent environmental perturbations compete for cofactors availability to regulate gene expression. To investigate the problem, we selected the estrogen and heat shock responses since the downstream transcription factors, the estrogen receptor (ER) and heat shock factor 1 (HSF1), were occupying mostly distinct genomic regions. Then, we defined communities of connected genes using Pol II Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) data to identify differentially occupied genes part of the same putative transcriptional ecosystem. Time-course responses following combinations of estrogen and heat shock treatments were used to measure recruitment of cofactors at target genes by chromatin immunoprecipitation (ChIP) and RNA levels by RT-qPCR. Our results support the existence of a limited pool of cofactors essential to respond to environmental disturbances. We propose the model that the physical proximity between genes combined with mobile, but limited transcriptional resources, and the capacity to share resources make the establishment of a transcriptional ecosystem a real possibility.

[102] MCGILL EPIGENOMICS MAPPING CENTRE: UPDATE ON MANDATE AND CAPABILITIES

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The Epigenomics Mapping Centre (EMC) and Epigenomics Data Coordination Centre (EDCC) at McGill University was established in 2012 by the CIHR Canadian Epigenetics, Environment, and Health Research Consortium (CEEHRC) initiative as one of two national hubs in Canada to support large-scale human epigenome mapping for a broad spectrum of cell types and diseases and provide reference epigenome maps for the larger International Human Epigenome Consortium (IHEC) effort. The successful, 1st phase of EMC produced 105 reference epigenomes and more than 400 partial reference epigenomes and contributed to ~50 publications in epigenome mapping. The CEEHRC phase 2 renewal (2017-22) allows the McGill EMC to continue its activities as a mapping core facility for collaborators and intramural projects. A new mandate for the mapping centre renewals is the promotion of epigenetic research within Canada by reserving 30% of the funded mapping capacity to cover the cost of profiling samples nominated and provided by the epigenetics research community. This community access program has generated high level of interest and top-ranked samples and projects will be selected for further analysis.
The EMC platform consists of data collection pipelines where wet-lab and bioinformatics resources are brought together in order to produce high quality epigenomic maps, available via controlled-access through the IHEC data portal leveraging Compute Canada high-performance computing resources. Reference epigenome generation prioritizes assays that are applicable to diverse cell populations and tissues: Whole Genome Bisulfite Sequencing (WGBS), RNA-Seq, and histone modification using chromatin immunoprecipitation (ChIP-Seq) with validated antibodies against a standard panel of histone marks (H3K27ac, H3K4me1, H3K4me3, H3K27me3, H3K9me3 or H3K36me3). ATAC-Seq (Assay for Transposase Accessible Chromatin combined with sequencing), a method for mapping genome-wide chromatin accessibility which uses a hyperactive Tn5 transposase to fragment DNA and integrate sequencing adapters into accessible regions of chromatin, is the most recent addition to the data collection pipeline. The easier assay workflow combined with a lower requirement of cell numbers

makes ATAC-Seq a viable replacement for some of the ChIP-Seq histone marks.
The McGill EMC is continuing to explore this as well as other assays (e.g. ChIPmentation) and protocol optimizations in order to address the challenges of more difficult samples (e.g. low cell number) and larger sample sizes, with the overall goal of improving efficiency and the quality of results.

[103] DECIPHERING THE ENHANCER SEQUENCE CODE OF PLURIPOTENT EMBRYONIC STEM CELLS USING COMPARATIVE EPIGENOMICS

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Mammalian genomes are 97-99% non-coding and the function of most of this DNA remains unknown. Enhancers are a major component of the non-coding genome, functioning to regulate gene expression in a tissue specific manner by binding transcription factors (TFs). Enhancers play a major role in evolution, disease and development; however, their identification is challenging due to variable location from their target gene and the non-significant increase in overall sequence conservation compared to surrounding non-coding sequences. Many of the critical TFs required to maintain the embryonic stem (ES) cell regulatory network are known, however, we do not currently understand how many and which specific TFs are required to build a functional enhancer. CRISPR deletion experiments of multiple TF bound loci (MTL) near Sall1 revealed discrepancies in enhancer functionality of MTLs indicating missing enhancer features exist that confer activity. A computational method was devised to identify the sequence code that confers enhancer activity in ES cells by decoding regions with conserved enhancer chromatin features (CHEF: MTL with high histone H3 K27 acetylation) in both mouse and human ES cells. CHEF regions are highly enriched in validated enhancers and depleted in tested regions that displayed no enhancer activity. Machine learning was applied to regions bound by at least one TF in mouse ES cells to determine the sequence features that distinguish CHEF regions in mouse and human from Conserved Low Enhancer Feature (CLEF) regions. Analysis of CHEF compared to CLEF regions revealed higher purifying selection pressure for transcription factor binding motifs (TFBM). To rank the importance of specific TFBM required to build an ES cell enhancer, LASSO (least absolute shrinkage and selection operator) was used to identify the TFBM most often present and conserved in CHEF regions. LASSO identified TFBM for TFs known to be involved in ES cell pluripotency maintenance as well as for TFs not yet characterized in this context. Mutagenesis of novel TFBM in a luciferase reporter vector revealed these do contribute to enhancer activity. Comparative sequence analysis in CHEF regions of 5 species revealed 14 as the average number of TFBM required to build a functional enhancer, however, there is flexibility in TFBM usage. To test this flexibility, we introduced multiple TFBM into an MTL lacking enhancer activity. Introduction of different subsets of TFBM conferred enhancer function, while loss of TFBM already present resulted in loss of gained enhancer function. Our analysis revealed a larger and flexible repertoire of TFBM used to build a functional enhancer than previously considered. Although many TFBM within conserved enhancers are conserved across species this flexible repertoire allows for sequence evolution without loss of function that can fine tune regulatory control.

[104] HISTONE MODIFICATIONS: FINDING THE RIGHT ANTIBODY FOR THE RIGHT APPLICATION

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Histones are decorated by post translational modifications (PTMs) that serve as epigenetic signatures for gene expression. These marks are essential for organizing the genome into active regions of euchromatin where the DNA is accessible and silenced regions of heterochromatin where the DNA is more tightly compact. Antibodies are employed for many of these studies and specificity is essential to confidently understand the location of specific histone modifications. Peptide arrays have traditionally been the gold standard for determining the suitability of these binding reagents as they provide valuable information regarding specificity for a large number of modifications especially in the context of neighboring modified residues. However, screening by array may be most analogous to applications such as western blot, where epitopes are displayed in a denatured environment. Increasing evidence suggests that peptide arrays do not recapitulate antibody performance in a ChIP experiment, due (in part) to the requirement for the antibody to recognize the modified histone within the context of an assembled nucleosome. As such, we are using multiple methods for accessing the specificity of a histone antibody in addition to peptide array. One method for validating in ChIP is to gain a picture of histone occupancy of multiple loci across a gene providing a more complete and comprehensive picture that the antibody is specific to its intended modification as particular modifications are known to be clustered at loci within a gene. Additional validation employs SNAP-ChIP™ (Sample Normalization and Antibody Profiling) to normalize ChIP experiments and rigorously test antibody specificity. Spiking-in a panel of recombinant semi-synthetic modified nucleosomes allows one to determine if an antibody is enriching the target of interest relative to other histone PTMs (i.e. off-target) in the panel. The current panel tests the specificity of methylated (K-Met) antibodies for mono-, di- and tri-methylation for H3K4, H3K9, H3K27, H3K36 and H4K20. Finally, peptide competition can be used to compete with an antibody for binding to demonstrate specificity for that modification or histone variant across applications. Our goal is to develop a comprehensive antibody portfolio comprising antibodies evaluated in the appropriate specificity test for a given application.

[105] IDENTIFYING BIOMARKERS FOR DRUG SENSITIVITY WITH A LATENT SPACE MODEL OF DNA METHYLATION

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Predicting drug sensitivity is a major challenge in personalized cancer medicine. The epigenetic landscape has been shown to have a complex relationship with gene expression and cellular phenotype, and disruption of the epigenome is pervasive in cancer. DNA methylation has an important, heritable role in regulating transcription - which in turn can present therapeutic targets - and presents an informative data set for predicting sensitivity to anti-cancer drugs. Using DNA methylation to inform drug sensitivity is a compromise among measurable cellular quantities because it is richer than mutation or copy number variation but more stable than gene expression. Furthermore, characterizing the epigenetic state through DNA methylation may provide both complementary and less transient information about cellular activity and vulnerabilities than gene expression.
The aim of this work is to assess how informative methylation state is of drug sensitivity to a wide range of targeted cancer drugs in cancer cell lines and identify predictive biomarkers for drug sensitivity. Previous work in epigenetic pharmacogenomics has used site-specific or region-based modeling methods that fail to account for the underlying relation among methylation sites, and methylation data remains largely unexplored as biomarkers of drug sensitivity.
We infer a latent space for DNA methylation data using principal component analysis (PCA) on the GDSC 1000 pharmacogenomic dataset to map data from the Illumina HumanMethylation450 array into a reduced space of hundreds of dimensions. The space of these methylation signatures or components is then used to predict sensitivity of cell lines to drug treatment. We demonstrate that this latent space is able to predict drug sensitivity better than single-site models and compare the predictive power to other methods like convolutional filters and bump hunting. Furthermore, the drug sensitivity predictions generalize across tissues. Further insights into the latent, covariant structure of methylation sites across a wide range of cellular contexts can increase the utility of epigenetic data for cancer treatment.

[106] MALE MICE DEFICIENT IN PRMT7 EXHIBIT PATERNAL IMPRINTING DEFECTS AT THE H19/IGF2 IMPRINTING CONTROL REGION

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Arginine methylation is a post-translational protein modification accomplished by a family of nine members (Blanc and Richard, 2017). They are mainly divided into two groups based on their methylation activity: Type I PRMTs (PRMT1, PRMT2, PRMT3, CARM1/PRMT4, PRMT6, and PRMT8) monomethylate and dimethylate arginine asymmetrically; Type II PRMTs (PRMT5 and PRMT9) monomethylate and dimethylate arginine symmetrically. PRMT7 is unique as it is the only PRMT which contains two AdoMet-binding motifs and likely has only arginine monomethylation activity (Miranda, Miranda et al. 2004, Zurita-Lopez, Sandberg et al. 2012), and therefore is classified as a type III PRMT.
In male germ cells, PRMT7 has been shown to interact with CTCFL (testis specific factor a transcriptional repressor) at the imprinting control region (ICR) of the paternal copy of the H19/Igf2 gene locus and regulate DNA imprinting in Xenopus oocytes (Jelinic, Stehle et al. 2006). The specific expression of the maternal H19 and paternal Igf2 is achieved by monoallelic methylation of the ICR located between the Igf2 and H19 genes (Thorvaldsen, Duran et al. 1998, Bell and Felsenfeld 2000). The interaction between PRMT7 and CTCF enhances the histone methyltransferase activity of PRMT7 at histone H4R3.
Here we show for the first time that conditional loss of PRMT7 in early primordial germ cells (TNAP-Cre), does not affect male fertility, but exhibit reduced DNA methylation at the H19/Igf2 imprinting control region (ICR) accompanied by an increase in H19 and a decrease in IGF2 expression level. Furthermore, by Chromatin ImmunoPrecipitation, we show that PRMT7 deficiency did not affect the recruitment of the de novo DNA methyltransferase 3A (DNMT3A) at the H19/Igf2ICR, as well as H19 and Igf2 promoter region suggesting that PRMT7 may regulate H19/Igf2 ICR methylation through other mechanisms.
These findings define PRMT7 as an important regulator of the H19/Igf2ICR methylation in the control of genomic imprinting and demonstrate that H19 paternal imprinting defects are not always correlated with male infertility.

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[107] THE HISTONE CHAPERONE ANP32E REGULATES H2A.Z EVICTION AND TURNOVER AND REGULATES MEMORY FORMATION IN THE HIPPOCAMPUS

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H2A.Z is a highly conserved variant of histone H2A, whose role as a memory suppressor we recently discovered (Zovkic et al. 2014). Specifically, H2A.Z is removed from chromatin after learning, and AAV-mediated H2A.Z depletion results in enhanced memory, suggesting that H2A.Z eviction promotes memory formation. However, the molecular mechanism underlying learning-induced H2A.Z removal remains uncharacterized. Studies in non-neuronal tissue recently identified Anp32E as an H2A.Z-specific histone chaperone that removes H2A.Z from nucleosomes, leading us to hypothesize that Anp32E-mediated removal of H2A.Z is crucial for memory formation. Here, we show that Anp32E and H2A.Z are simultaneously bound to several H2A.Z-enriched genes in the mouse hippocampus. In response to fear conditioning, H2A.Z and Anp32E are concurrently evicted from sites in which they co-localize. Moreover, Anp32E is functionally relevant for memory formation, as AAV-mediated knock-down of this chaperone in the hippocampus results in impaired memory, whereas Anp32E overexpression results in enhanced memory. Notably,

manipulating Anp32E levels in cultured neurons and in the hippocampus results in altered gene expression of H2A.Z-regulated genes and altered accumulation of H2A.Z in different chromatin fractions. In addition, knock-down of Anp32E in primary hippocampal neurons results in impaired dendritic branching. Strikingly, simultaneous knock-down of Anp32E and H2A.Z results in rescue of altered gene expression, impaired dendritic branching and memory formation seen in Anp32E knock-down. Overall, our data suggest that Anp32E is a functional component of the molecular machinery regulating H2A.Z eviction during learning and turnover in neurons.

[108] RECURRENT ONCOGENIC CHROMOSOMAL TRANSLOCATIONS INVOLVING THE NUA4/TIP60 AND PRC2 COMPLEXES LEAD TO ABERRANT CHROMATIN LANDSCAPE

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NuA4 (a.k.a. TIP60/p400) is an evolutionarily conserved multifunctional protein complex that possesses two catalytic functions – a histone acetyltransferase (HAT) activity and a histone exchange activity that deposits H2A.Z variant. The complex plays critical roles in transcriptional activation and DNA damage repair and is linked to a variety of human diseases including cancer (haplo-insufficient tumor suppressor). Recent genomic studies have identified recurrent chromosomal translocations in patients with endometrial stromal sarcomas (ESS) and ossifying fibromyxoid tumors (OFMT) leading to an in-frame fusion of four different NuA4 subunits (EPC1, P400, MEAF6, BRD8) to PHF1 (PCL1), a protein associated with the polycomb repressive complex 2 (PRC2) complex. In contrast to NuA4, PHF1 and PRC2 proteins are linked to transcriptional repression of key developmental genes through methylation of histone H3 on lysine 27 (H3K27) by the EZH2 methyltransferase (HMT).
We present the biochemical and functional characterization of the EPC1-PHF1 fusion. The chimeric protein becomes incorporated into the NuA4 complex in place of EPC1, but also retains the ability to associates with PRC2, creating a hybrid super-complex harboring both HAT and HMT activities. EPC1-PHF1 binding increased histone acetylation and H2A.Z incorporation within regions regulated by PRC2, notably within the HOXC and HOXD gene clusters. This is usually correlated with increased gene expression, consistent with the notion that EPC1-PHF1 mis-targets the HAT and histone exchange activities of NuA4 to chromatin regions that are normally maintained in the silenced state. Increased H3K27 methylation was also detected, albeit at much lower frequency, leading to decreased transcription. Interestingly, bivalent chromatin regions harboring both H3K4me3 and H3K27me3 are preferred targets of EPC1-PHF1. Finally, mistargeting of histone acetylation on HOX clusters leads to loss of H3K27 methylation over entire specific topology-associated domains (TADs) without affecting the neighboring ones, albeit also bound by PRC2 complexes.
Two other recurrent translocations in ESS are also linked to PRC2 components, JAZF1-PHF1 and JAZF1-SUZ12. We show that the conserved transcription factor JAZF1 is in fact physically and functionally linked to NuA4 in vivo, recreating the same NuA4-PRC2 mega-complex as in the other translocations. Overall our data support the hypothesis that NuA4-PRC2 fusion promotes oncogenesis by joining two chromatin modifying activities with diametrically opposite functions, subverting the activities of the NuA4/TIP60 complex and leading to the deregulation of PRC2 target regions. These findings further underline the importance of deregulated epigenetic modifiers in promoting oncogenesis, in part through changes of the chromatin landscape over large genomic regions.

[109] TOWARDS NUTRITIONAL AND EPIGENOMIC INTERVENTIONS IN PROSTATE CANCER PREVENTION AND MANAGEMENT

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Prostate cancer (PCa) affects millions of men each year worldwide with incidence increasing in elderly male. With life expectancy on the rise, PCa is rapidly becoming an important social and economic burden for the Canadian health system. Preventing or delaying progression of a significant proportion of these cancers would be a major achievement.
Epidemiological studies support that environment, and most importantly diet, influences the onset and evolution of PCa. We conducted a phase IIb randomized controlled trial for a new active surveillance (close monitoring without invasive procedures) protocol involving a dietary intervention, compared to the standard of care dutasteride, for men with low-grade PCa. Prostatic biopsies, blood and urine were collected all along the clinical trial. Our clinical outcome shows that a diet rich in omega-3 fatty acids is an effective intervention to block low-grade PCa progression toward high-grade lethal cancers. While our clinical study provides a strong signal for the effect of an omega-3-rich diet in reducing the short-term incidence of high-grade cancer, it also highlights that many patients will not show benefit of such dietary intervention. Here we are leveraging this unique clinical trial to determine which patients are at risk of high-grade PCa, with the long-term goal to optimize dietary interventions for patients with a low-grade PCa as an alternative to invasive interventions. To do so, we are conducting in depth gene expression profiling using RNA-Seq to determine gene signatures associated with response and non-response to a dietary intervention in addition to risk of high-grade PCa progression. Furthermore, we are correlating gene expression changes with chromatin accessibility using ATAC-Seq to understand how an omega-3-rich diet intervention is translated

molecularly. This will identify potential targetable synergistic pathways to further improve the beneficial preventive effect of the diet.
By leveraging our unique clinical study, we aim at creating a stratification method to determine the likelihood of a patient with low-grade PCa to progress toward a high-grade lethal PCa. Furthermore, we are providing a first look into the epigenomic molecular mechanisms explaining the response and nonresponse to a dietary intervention.

[110] BIOINFORMATIC ANALYSIS OF MOUSE PFC DNA METHYLATION IN RESPONSE TO ACUTE, SUB-CHRONIC AND CHRONIC NEUROPATHIC PAIN

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Aim of Investigation: Chronic pain is associated with the functional and anatomical remodeling of prefrontal cortex (PFC) in both human patients and rodent models. The PFC is commonly implicated in pain modulation and pain-related comorbidities. Following therapeutic intervention, these changes can be partially reversed. Understanding the mechanisms underlying recovery of cortical function may provide insights into chronic pain's pathobiology and reveal new treatment approaches.
As a reversible regulator of gene expression that is responsive to environmental effects, DNA methylation is an ideal candidate mechanism to mediate PFC plasticity in response to chronic pain. We have previously shown PFC DNA methylation undergoes significant changes 6 months post-spared nerve injury (SNI) and results in the differential methylation of thousands of PFC genes.
The current study used epigenome-wide methylation analysis to track changes in the PFC post-SNI at acute, sub-chronic and chronic time points and to identify pain-related genes and functional pathways over time. We hypothesize that DNA methylation contributes to the development and maintenance of chronic pain over time by mediating changes in PFC gene expression.
Methods: Male six week old CD-1 mice underwent SNI or sham surgery and PFC was harvested from Sham or SNI at 2-days, 2-weeks or 6-months post-injury. DNA was bisulfite-converted and sequence-capture acquired genes for Illumina sequencing to determine DNA methylation levels. Bioinformatic analyses determined differential methylation and functional pathway analysis was performed to identify pain-relevant genes related to chronic pain development and maintenance.
Results: DNA methylation changes were observed in hundreds of genes, comparing SNI and sham animals at acute, sub-chronic and chronic time points. Examples include differential methylation of methyl-CpG binding domain protein 4 (Mbd4) in 6-month post-SNI versus 6-month sham animals. The chronic time point has the greatest number of differentially methylated genes.
Discussion/Conclusions: Identifying pathways unique to time point or injury condition may reveal specific mechanisms regulating chronic pain's development and maintenance over time, allowing for a better understanding of the overall response to chronic pain. Determining the key regulatory pathways or epigenetic mechanisms underlying chronic pain may provide novel intervention targets or therapies that better account for the complex nature of chronic pain.

[111] PHYLOGENETIC DIVERSIFICATION OF SUV420H2 FUNCTION

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Recent evidence indicates that spatial partitioning of pericentromeric heterochromatin reflects phase separation (aka as liquid-liquid demixing), a process whereby protein ensembles coalesce into droplets in an otherwise aqueous environment. Nevertheless, there are considerable differences in the organization of pericentromeric heterochromatin between species that are difficult to reconcile with phase separation. Trimethylation of lysine-9 on histone H3 (H3K9me3) and lysine-20 on H4 (H4K20me3) establish the characteristic chromatin features at the pericentromere. These arise through a pathway that involves the lysine methyltransferase enzymes SUV39H1/2 and SUV420H1/2, and the chromobox proteins CBX1/3/5 (aka HP1ß/γ/α). The paradigm of pericentromere phase separation, however, has been established solely with analyses of CBX5 in mouse cells, where pericentromeres from multiple chromosomes assemble into larger chromocentres. Based on a high degree of sequence conservation amongst CBX5 homologs, it is anticipated that its phase separation potential is retained in diverse eukaryotes. In contrast, this is not clear for SUV420H2, which is only found in the Tetrapoda superclass. Moreover, the region responsible for SUV420H2 localization to the pericentromere is only present in mammals and displays considerable divergence within this class. Phylogenetic analyses therefore indicate that CXB5 and SUV420H2 are under distinct evolutionary pressures, which has important implications for understanding how they coordinate pericentromere organization and phase separation. Our ongoing analyses therefore support a model in which SUV420H2 employs different strategies to spatially partition pericentromeric heterochromatin with the potential to reveal fundamental aspects of chromatin biology and its evolutionary diversification.

[112] DNA METHYLATION AND THREE DIMENSIONAL CHROMATIN STRUCTURE OF THE IRXA GENE CLUSTER IN HUMAN COCAINE DEPENDENCE.

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Background: Multiple, interacting biological mechanisms are likely to contribute to the development and maintenance of cocaine use disorders. Epigenetic mechanisms are of particular interest as they may mediate the long-term effects of chronic cocaine abuse on brain cell functioning in addiction-related brain circuits. Although animal research has built a strong case for the role of DNA methylation and chromatin remodelling in cocaine dependence, little is known about the relationship between these phenomena in human patients.
Methods: We used reduced representation bisulfite sequencing (RRBS) to identify genome-wide differential methylation, in post mortem caudate nucleus and nucleus accumbens from 29 individuals with cocaine dependence and 29 drug-naïve controls. We validated and replicated our findings and using targeted bisulfite sequencing, and used fluorescence activated nuclei sorting (FACS) to investigate cell-type specificity. We also used transcriptome sequencing, and chromatin conformation capture (3C) based techniques to explore the genomic regulation of the IRXA gene cluster. Our in vitro studies use immortalized fetal midbrain cells (RenCells) and dCas9-DNMT3a epigenome editing to investigate causal relationships between cocaine-related methylation changes and chromatin functioning at this locus.
Results: We found altered methylation within two genes of the neurodevelopmental gene cluster, IRXA, in the caudate nucleus. We replicated our finding of decreased methylation within a cluster of 21 CpGs in the gene body of IRX2 in an independent cohort, and found this effect to be specific to neuronal nuclei. In addition, cocaine dependence is associated with increased expression of the IRX2 gene and altered co-expression of IRX1 and IRX2 transcripts. Moreover, we identified a large chromatin loop in human cells, which is related to IRX2 methylation levels and is likely involved in IRXA regulation. Preliminary work suggests that methylating this locus in vitro results in significantly reduced expression of both IRX1 and IRX2, which is likely mediated through altered chromatin looping.
Conclusions: Chronic cocaine dependence is associated with widespread alternations in DNA methylation in the human striatum, including decreased methylation within the gene body of IRX2 in the caudate nucleus. This appears to be associated with dysregulated gene expression and may be driven by altered chromatin looping. Further studies will continue to investigate the relationship between multiple epigenetic mechanisms in human cocaine use disorders.

[113] COMPARATIVE EPIGENOMICS REVEALS THE CONTRIBUTION OF MULTIPLE TRANSPOSABLE ELEMENT SUBFAMILIES TO INFLAMMATORY CYTOKINE-INDUCED NF-KB BINDING

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Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is an essential and evolutionarily conserved transcription factor involved in innate immunity and inflammation. During inflammation, NF-κB rapidly translocates to the nucleus, binds to cis-regulatory elements (CREs), and drives target gene expression. Transposable elements (TEs) have been recently shown to innovate immune regulatory networks during evolution by propagating CREs across the genome. However, the extent to which TEs have contributed functional NF-κB binding sites to mammalian genomes is not known. Here we characterized the contribution of TEs to NF-kB binding sites in response to TNFα (Tumor Necrosis Factor alpha) in primary aortic endothelial cells obtained from human, mouse and cow. We found that 55 TE subfamilies were enriched within NF-kB binding sites in at least one species. The majority of these subfamilies were derived from lineage specific endogenous retroviruses (ERVs). These NF-kB bound ERVs possess multiple active epigenetic marks and were significantly distributed near TNFα-induced genes. We also found seven ancient TE subfamilies that were enriched in at least two species including the mammalian DNA transposon MER81. Ancestral MER81 sequences possess two intact NF-kB binding motifs and the loss of one or both of these motifs correlated with loss of NF-kB binding. Human NF-κB bound MER81 elements showed active enhancer-like characteristics and were associated with functionally annotated immune genes. Taken together, our results demonstrate the potential involvement of TEs in establishing NF-kB mediated immune regulatory networks during mammalian evolution.

[114] BAF COMPLEX MUTATIONS DYSREGULATE ENHANCER LANDSCAPES IN MALIGNANT RHABDOID TUMOR AND OVARIAN CLEAR CELL CARCINOMA

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Background: Genetic mutations to members of the mSWI/SNF(BAF) complex are recurrent events in cancer and are thought to contribute to carcinogenesis. Cancer subtypes show remarkable specificity to BAF 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). BAF complexes play important roles in transcriptional regulation but the drivers of selection for specific subunit disruption in cancer subtypes is currently unknown.
Methods: To investigate the role of SMARCB1 and ARID1A in MRT and OCCC, we performed ATAC-seq, native ChIP-seq (H3K4me3, H3K4me1, H3K27ac, H3K27me3, H3K36me3, H3K9me3) for histone modification, whole genome bisulfite and RNA-seq on MRT and OCCC isogenic cell line models: the MRT cell line G401 engineered to re-express SMARCB1 (G401-B1), and an OCCC line engineered with a biallelic loss of ARID1A (AC14).
Results: In our MRT model we identified 3269 and 2398 open chromatin regions in the presence and absence of SMARCB1 respectively, of which 1624 (49.7%) were found only in the presence of SMARCB1. Moreover, we identified an increase in the number of active enhancers, as defined by the presence of the H3K27ac and H3K4me1, in the presence of SMARCB1. We observed CpGs within G401 unique enhancers to be

hypomethylated, whereas G401-B1 unique enhancers were hypermethylated. Lhx2, ERRA, FOXO1 and SMAD3 are the most significantly enriched binding elements in the SMARCB1 specific enhancers and gene enrichment analysis of transcripts up-regulated in G401-B1 cells compared with G401 showed enrichment in genes implicated in cell morphogenesis and differentiation.
In contrast loss of ARID1A did not result in a global change of chromatin accessibility with equivalent gains and losses of open chromatin regions observed in the presence and absence of ARID1A. Instead, ARID1A loss altered chromatin accessibility in a context specific manner, largely at active enhancers (H3K27ac marked) but not primed enhancers (H3K4me1 marked). ARID1A loss was also associated with a decrease in the number of super-enhancers and motif analysis of ARID1A dependent super-enhancers revealed enrichments for ATF3, AP-1, BATF and Jun-AP1 motifs, as well as tissue specific transcription factors such as RUNX, MafK and Bach2.
Conclusion: Our analysis revealed that SMARCB1 and ARID1A loss is associated with subunit specific genome-wide chromatin dysfunction leading to distinct enhancer alterations and differential gene expression.

[115] TET2-DEPENDENCE OF VITAMIN C INDUCED EPIGENOMIC ALTERATIONS IN ACUTE MYELOID LEUKEMIA

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Neomorphic mutations in isocitrate dehydrogenase 1/2 (e.g. IDH1 R123H) and heterozygous inactivating mutations in Ten Eleven Translocation 2 (TET2) methylcytosine (5mC) dioxygenases are mutually exclusive events in de novo acute myeloid leukemia (AML) and account for more than 40% of AML cases. IDH1/2 mutations drive epigenomic dysfunction by producing the oncometabolite R-2-hydroxyglutarate (R-2HG), which inhibits TET2, and is associated with alterations in the methylation levels of CpGs genome-wide. Previously, we established that vitamin C (vitC) induces epigenetic reprogramming through TET activation in an engineered murine leukemic model overexpressing HOXA9 and harbouring a neomorphic mutation in IDH1 (R132H). To further understand the role of TET2 in this reprogramming we performed meDIP-seq and hmeDIP-seq in a murine AML model expressing IDH1 R132H in which Tet2 had been genetically inactivated by CRISPR/Cas9 (TET2KO). TET2KO induced a selective advantage in our cell line over multiple passages. Examination by immunohistochemistry of the TET2KO line following exposure to 0.3mM vitC treatment for 12 hrs showed a reduction in total 5hmC gain compared to the parental line. Analysis of the hmeDIP-seq datasets generated following vitC treatment showed induced local gains in 5hmC in both R132H and TET2KO specifically at regulatory regions previously annotated to be marked with H3K4me1 marks in R132H, though to a lesser extent in the TET2KO line. This specific gain in 5hmC was also observed in R132H with a lower dose of vitC (0.1mM), but not in the TET2KO line. Regardless of TET2 status, vitC induced a directional gain in 5hmC at regions shown to be hypermethylated by mutant IDH1. These common regions were associated with genes implicated in apoptosis and hemopoiesis and were enriched in PU.1 binding sites, while regions uniquely hydroxymethylated in R132H were associated with genes implicated in DNA repair mechanisms. Taken together, this suggests a model in which TET3, which is expressed at similar levels in AML, may perform some redundant functions similar to TET2 after vitC induction.

[116] DECIPHER THE ACTIVATION MECHANISM OF CFP1/CPS40 ON SET1-LIKE COMPASS

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Methylation of histone 3 on lysine-4 (H3K4) is a well-known post-translational modification that is catalyzed by the lysine Methyl-Transferase 2 (KMT2) family of enzymes. KMT2 enzymes contain a conserved catalytic SET domain and function as a complex referred to as the COMPlex ASsociated to SET1 (COMPASS). While COMPASS complexes share common core composed of WDR5/Cps30, RbBP5/Cps50, Ash2L/Cps60 and DPY30/Cps25 (WRAD); they have different abilities to methylates H3K4 to different states (mono-, di-, tri-methylation) and targets it to distinct genome locations. One special COMPASS subclass, SET1-like (including yeast SET1, human KMT2G and F), is responsible for trimethylation of H3K4 at open and potentially active promoters. One hypothesis is that specific subunits may modulate the catalytic activity of the core COMPASS complex in a context-dependent manner[1]. Cfp1/Cps40 is a unique subunit of SET1-like COMPASS and is a key to proper H3K4 trimethylation. Cells that lack Cfp1/Cps40 Cps40 have lost 80% of global H3K4 trimethylation but have no significant changes in H3K4 mono- and di-methylation[2,3]. In order to understand how Cfp1/Cps40 regulates SET1, we identified and examined the interacting sites on Cfp1/Cps40 using X-crystallography and performed a kinetic characterization for the role of Cfp1/Cps40 role in progressive catalysis.

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[117] HEART ENHANCERS WITH DEEPLY CONSERVED REGULATORY ACTIVITY ARE ESTABLISHED EARLY IN DEVELOPMENT

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During the phylotypic period embryos from different genera show similar gene expression patterns, implying common regulatory mechanisms. To identify enhancers involved in the initial events of cardiogenesis, which occurs during the phylotypic period, we isolated early cardiac progenitor cells from zebrafish embryos and characterized 3838 open chromatin regions specific to this cell population. Of these regions, 162 overlapped with conserved non-coding elements (CNEs) that also mapped to open chromatin regions in human. Most of the zebrafish conserved open chromatin elements tested drove gene expression in the developing heart. Despite modest sequence identity, human orthologous open chromatin regions could recapitulate the spatial temporal expression patterns of the zebrafish sequence, potentially providing a basis for phylotypic gene expression patterns. Genome-wide, we discovered 5598 zebrafish-human conserved open chromatin regions, suggesting that a diverse repertoire of ancient enhancers is established prior to organogenesis and the phylotypic period.

[118] NONCODING MUTATIONS TARGET CIS-REGULATORY ELEMENTS OF FOXA1 IN PRIMARY PROSTATE CANCER

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Prostate cancer is the second-most commonly diagnosed malignancy among men worldwide. Exome sequencing efforts revealed that FOXA1 is recurrently mutated in more than 2% of primary and 12% of metastatic prostate cancer patients. Functioning as a pioneer transcription factor, FOXA1 recruits and facilitates the binding of AR to chromatin, promoting target gene expression programs for prostate cancer growth and progression. Despite the oncogenic role FOXA1 plays in prostate cancer etiology, the cis-regulatory network underpinning its expression remains unclear. Leveraging essentiality datasets, we observe and validate that FOXA1 is essential for prostate cancer cell proliferation. Integrating genomic and epigenomic datasets, we identified a set of 6 FOXA1 cis-regulatory elements (CREs) that contributes to its regulatory network upon prioritization based on the presence of single-nucleotide variants (SNVs). Deletion of each CRE resulted in significant reduction FOXA1 expression, with further reduction observed upon subsequent deletion of an additional CRE. Notably, deletion of the CREs also resulted in significantly lower cell proliferation, in accordance to the oncogenic properties of FOXA1. Interrogation into the 10 SNVs that map to these CREs through luciferase assays further revealed that 6 of the 10 SNVs demonstrated gain-of-function potential as the mutant allele drove significantly higher luciferase activity compared to the wild-type allele.
Taken together, we provide the first description of a set of 6 CREs contributing to the FOXA1 cis-regulatory network targeted by gain-of-function SNVs. Combining recent reports that show the loci harboring these CREs as target of tandem duplication in metastatic prostate cancer, it warrants further investigation of the FOXA1 cis-regulatory network in prostate cancer progression. More broadly, our work emphasizes the importance of unveiling cis-regulatory networks that converge on the same target gene, and the need to interrogate the function of genetic alterations targeting CREs.

[119] TANDEM DUPLICATION LEADS TO REWIRING OF ENHANCER-GENE INTERACTIONS IN THE PATHOGENESIS OF QUEBEC PLATELET DISORDER

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Organization of the genome into topologically associated domains (TADs) and TAD sub-structures (sub-TADs) is essential for gene regulation, with emerging examples of human disease caused by disruptions in TAD architecture. Quebec platelet disorder (QPD) is an inherited bleeding disorder characterized by ectopic overexpression of urokinase plasminogen activator (uPA) in megakaryocytes (MKs) and > 100-fold increases in uPA in differentiated MKs and platelets. The causal mutation of QPD is heterozygous tandem duplication of a 78kb region containing PLAU, the gene encoding uPA. However, the magnitude and tissue-restricted pattern of PLAU overexpression in QPD implicate a regulatory defect beyond copy-number change. Aiming to establish the molecular mechanism of QPD, we profiled the epigenetic and chromatin interaction landscape of the PLAU locus in granulocytes and MKs derived from QPD and control individuals. Profiling of active histone marks shows that QPD does not alter the active enhancer landscape at the PLAU locus in QPD versus control MKs. Instead, we found that the QPD duplication spans a CTCF-associated sub-TAD and positions the duplicated copy of PLAU within the regulatory domain of a highly conserved hematopoietic enhancer. Profiling of chromatin contacts by 4C-seq reveal that this CTCF-associated sub-TAD normally isolates PLAU from other regulatory elements in the locus and may be important for silencing of PLAU under normal conditions. Taken together, these findings suggest that pathogenicity in QPD results from alteration in regulatory architecture and ectopic activation of PLAU by a lineage-restricted enhancer. Our findings represent a unique case of human disease involving disruption of regulatory architecture at the sub-TAD scale and emphasize the importance of evaluating tissue-specific genome regulatory architecture in interpreting the pathogenic mechanisms of other diseases involving structural variation.

[120] ESTIMATION OF TRANSCRIPTION FACTOR DYNAMICS ACROSS PUBERTAL DEVELOPMENT IN THE MOUSE HYPOTHALAMUS

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Puberty is defined as the period when adults reach sexual maturity and can produce gametes. The activation of puberty is controlled by the hypothalamic-pituitary-gonadal axis, stemming from the hypothalamus. Alterations in pubertal onset lead to complications in diseases such as cardiovascular disease, diabetes, and mental health. Furthermore, pubertal onset leads to a large amount of changes in gene expression, which is controlled by alterations in transcription factor (TF) activity. To investigate hypothalamic pubertal regulation, we measured gene expression and epigenetics through RNA-seq, and ChIP-seq in H3K4me2, H3K27me3, H3K27ac, and H3K36me3 before and after puberty in the hypothalamus of male and female mice. We used chromatin state, nucleosome repositioning, and motif enrichment analysis to identify a set of “dynamically upregulated” and “dynamically downregulated” genes across puberty. Then, we tested over-representation of TF binding to identify TFs which may be responsible for the changes in gene regulation. Finally, we validated these identified TFs by having them independently enrich for differentially expressed genes across puberty. Thirteen TFs passed all thresholds, one of which is *Egr1,* which plays a functional role in puberty in the pituitary gland and is highly expressed in the human hypothalamus. Furthermore, *Egr1* is also differentially expressed in the mouse hypothalamus across puberty. We are currently completing ChIP-seq of Egr1 to investigate its differential binding across pubertal development.

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