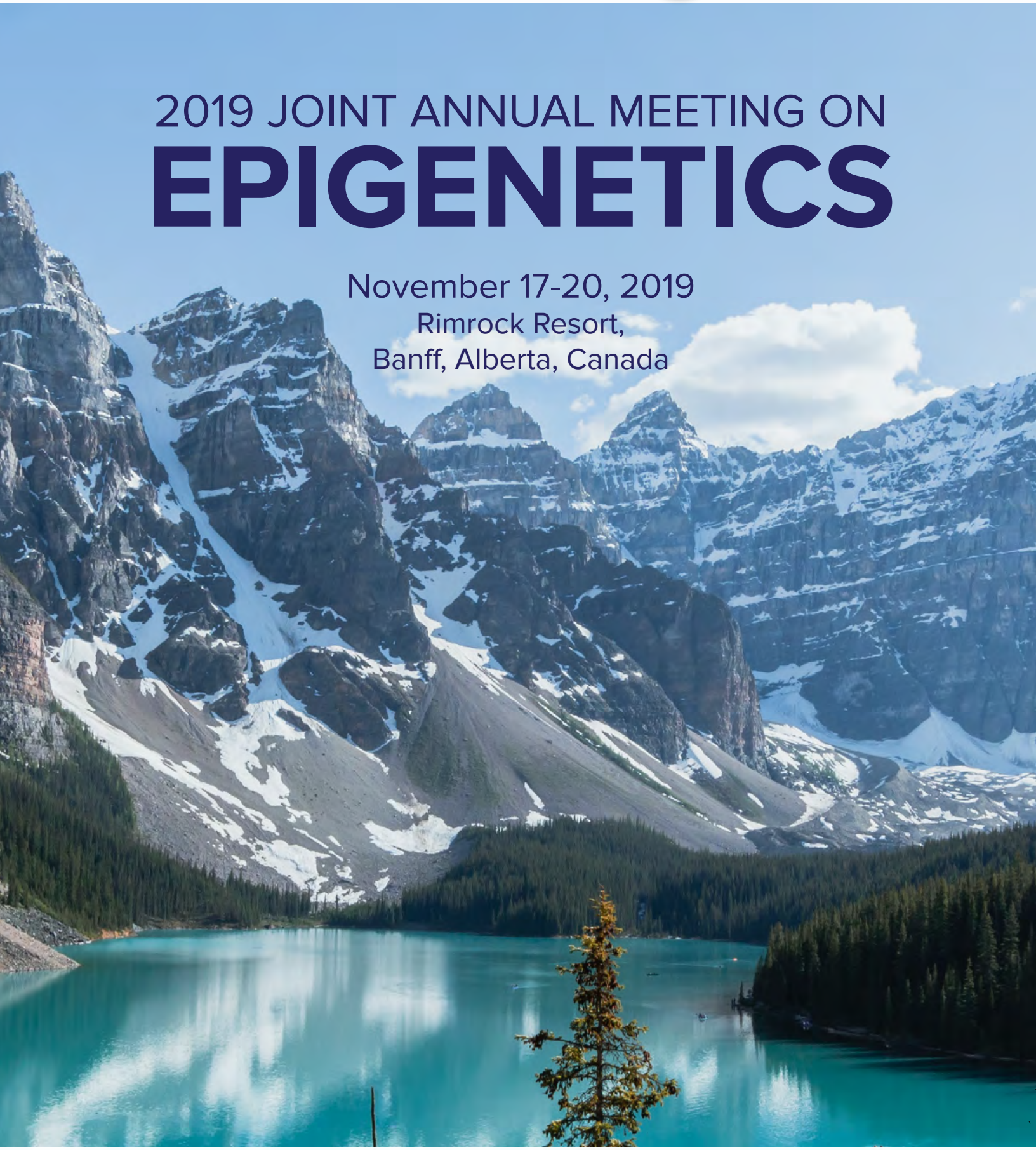
CEEHRC NETWORK
Canadian Epigenetics, Environment and Health Research Consortium Network

IHEC
International Human Epigenome Consortium

2019 JOINT ANNUAL MEETING ON EPIGENETICS

November 17-20, 2019
Rimrock Resort,
Banff, Alberta, Canada



CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

GenomeCanada

GENOME ALBERTA

Welcome!

Dear Colleagues,

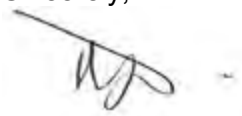
It is a pleasure to welcome you to the Rimrock Resort in beautiful Banff Alberta for the 6th Annual Canadian Epigenetics and Environment Health Research Consortium Epigenetic Conference being held in partnership with the International Human Epigenome Consortium Annual General Meeting.

This year we welcome over 260 national and international epigenetics researchers to share their research and to engage in dialogue on the current state of epigenetics research. Our scientific program includes distinguished invited speakers in the fields of epigenetics and epigenomics covering topics including epigenomic measurements at both the bulk and single cell level, mechanisms of epigenetic regulation, chromatin organization and epigenetic dysfunction in disease. Interleaved throughout the meeting will be talks highlighting contributions of the International Human Epigenome Consortium in advancing international epigenomic research and collaboration. These topics will be covered over 10 sessions that include keynote and plenary lectures, technical talks, rapid fire oral presentations, an epigenetics study design workshop, and 2 poster sessions. As in previous years, we hope that the meeting format will encourage interaction and networking between epigenetic researchers across career stages while providing a respectful environment that stimulates scientific debate and catalyzes new collaborations.

This year we are also delighted to announce renewal of funding for the CEEHRC Network. The aim of the CEEHRC Network is to support and advocate for epigenetic research in Canada, accelerate its translation, and facilitate access to state-of-the-science epigenomic mapping tools and curated reference human epigenomic datasets. During this meeting, the Network will be launching a public outreach initiative that aims to provide evidence-based interpretations of epigenetics research and its implications to the public. To catalyze this knowledge translation effort and engage you in this important science literacy initiative we will be asking you to reflect on your definition of epigenetics during the meeting. More information about the Network and its goals can be found at our website: www.epigenomes.ca.

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

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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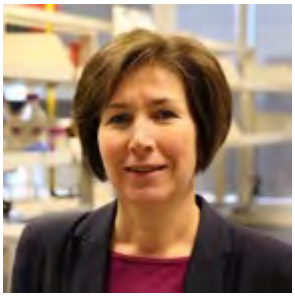
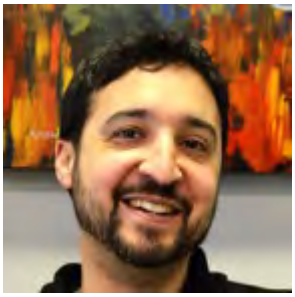
Organizing Committee



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 Speakers

Bing Ren
University of California San Diego

Dr. Ren is Director of the Center for Epigenomics and Professor of Cellular and Molecular Medicine at the University of California, San Diego (UCSD). He is also a Member of the Ludwig Institute for Cancer Research (LICR). Dr. Ren obtained his Ph.D. in Biochemistry from Harvard University in 1998 and joined the faculty at LICR and UCSD in 2001, after completing postdoctoral training at the Whitehead Institute. Dr. Ren is studying how the non-coding sequences in the human genome direct spatiotemporal patterns of gene expression, how epigenetic mechanisms regulate their output during development, and how changes in these sequences contribute to human diseases. Specifically, he focuses on the identification and characterization of a class of transcriptional control elements known as enhancers. His lab has developed transformative tools for genome-wide analysis of enhancers and elucidated the chromatin features of these regulatory sequences. He is a recipient of the Chen Award for Distinguished Academic Achievement in Human Genetic and Genomic Research, and an elected fellow of the American Association for the Advancement of Science.

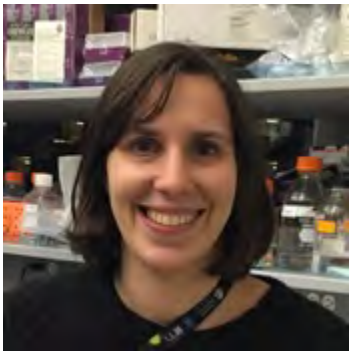
Ellen Rothenberg
Caltech

Ellen Rothenberg is the Albert Billings Ruddock Professor of Biology at Caltech. She studies gene regulation and development of T lymphocytes, gene networks controlling hematopoietic cell fates, and mechanisms underlying the dynamics of single-cell developmental decisions. She graduated from Harvard University, earned her Ph.D. from Massachusetts Institute of Technology, and was a Jane Coffin Childs Postdoctoral Fellow at Memorial Sloan-Kettering Cancer Center. After an assistant professorship at the Salk Institute for Biological Studies, she joined the Caltech faculty in 1982. Her honors include the Richard P. Feynman Prize for Excellence in Teaching (2016), and election as Fellow of the American Association for the Advancement of Science (2017), Fellow of the American Academy of Arts and Sciences (2018), and Distinguished Fellow of the American Association of Immunologists (inaugural class, 2019). She serves on multiple editorial and scientific advisory boards and has organized many international conferences in immunology and systems developmental biology.

John Stamatoyannopoulos
University of Washington

Dr. Stamatoyannopoulos' laboratory uses high-scale molecular, computational, and genome engineering technologies to decode the regulatory circuitry of the human and other complex genomes. Major ongoing efforts are (i) to create comprehensive atlases of regulatory DNA encoded in the human and mouse genomes; (ii) to define regulatory networks that control cell fate and response; (iii) to identify and characterize human regulatory variants associated with common human diseases; and (iv) to develop next-generation technologies for interrogating, interpreting, and programming the human regulatory genome. Dr. Stamatoyannopoulos holds degrees in Biological Sciences, Symbolic Systems, and Classics from Stanford University, and an M.D. from the University of Washington School of Medicine. Dr. Stamatoyannopoulos completed his medical training at Harvard Medical School, including internship and residency in Internal Medicine at Brigham and Women's Hospital, and fellowship in Oncology and Hematology at the Dana Farber Cancer Institute and Massachusetts General Hospital.

Invited Speakers



Julie Brind'Amour
University of British Columbia

Dr. Brind'Amour is a research associate at UBC in the laboratory of Dr. Matthew Lorincz. Her research has focused on studying how the interplay between transcription, histone modifications and DNA methylation impacts epigenetic remodeling in germ cells and in early embryonic development.

Judy Cho
Mount Sinai

Dr. Judy H. Cho, MD, is the Director of the Charles Bronfman Institute for Personalized Medicine (CBIPM), and the Ward-Coleman Chair for Translational Genetics at the Icahn School of Medicine at Mount Sinai. Her research focuses on inflammatory bowel disease (IBD) genetics and disease mechanisms and her laboratory is applying single cell RNASeq and CITE-Seq toward developing novel therapeutic insights. Cell-cell communications within the intestinal wall likely underlie a substantial fraction of pathophysiologic heterogeneity, especially immune cell to stromal cell interactions. Additional heterogeneity is driven by uncommon risk alleles, notably NOD2 in Crohn's disease, found exclusively within European ancestry populations. Dr. Cho has served as Principal Investigator of the Data Coordinating Center of the NIH-funded NIDDK IBD Genetics Consortium since 2003. In this capacity, she has led efforts in the identification of over 200 genetic regions associated to IBD, notably the association of the IL-23 pathway to both Crohn's disease and ulcerative colitis. Since 2015, Dr. Cho has led the CBIPM, which includes the School's major biobank, named BioMe, now including over 50,000 patients consented for retrospective and prospective mining of the electronic health record. She is leading School efforts in organizing a secure DataCommons for BioMe-based datasets for broad use in both academic and industry collaborations. BioMe represents one of the most diverse biobanks in the world and sequencing results underscore the enormous potential of a genetics first strategy in clinical care. These initiatives reflect the School's major commitment to Personalized Medicine to improve the care of patients on an individualized basis.

Jacques Côté
Université Laval

Jacques Côté obtained his PhD from Laval University and did post-doctoral training at Penn State University where he identified and characterized chromatin remodeling complexes (SWI/SNF) involved in gene activation. He then joined Laval University where he currently holds the Canada Research Chair in Chromatin Biology and Molecular Epigenetics and is director of the Oncology Division of the CHU de Quebec Research Center. His research aim is to understand chromatin dynamics associated with gene regulation, DNA repair and replication. His group studies protein complexes that control acetylation and methylation of histones, and the composition of chromatin. They dissect the molecular mechanisms of epigenetics, in which signals to chromatin mark different genomic loci and are read by effectors to translate a biological response. Major discoveries in his laboratory characterized the structure and function of the NuA4/TIP60 acetyltransferase complex, identified recognition modules for the epigenetic histone signature and demonstrated the essential role of chromatin modifying activities in the processes of DNA repair and replication in eukaryotes.

Daniel De Carvalho
University of Toronto

Dr. De Carvalho is a senior scientist at Princess Margaret Cancer Centre and Associate Professor at the Department of Medical Biophysics at University of Toronto. Moreover, he holds the Canada Research Chair in Cancer Epigenetics and the Helen M Cooke endowed professorship. As recognition for his scientific excellence, dr. De Carvalho has received multiple awards, including the Canadian Cancer Society Bernard and Francine Dorval Prize, the CIHR new investigator salary award and the CIHR-ICR new Early Career Award in Cancer. Dr. De Carvalho was recently inducted into the Royal Society of Canada College of New Scholars, Artists and Scientists. His research was highlighted by ASCO in its annual report as one of the most important biomedical discoveries in oncology in 2012, by Nature Medicine (top 10 notable advances of 2015), NEJM (clinical implications of basic research: Epigenetic Modulators and the New Immunotherapies) and the Wall Street Journal. His research program focuses on the translational aspects of cancer epigenetics. He is motivated by the fact that DNA methylation profiles are largely changed between normal tissue and cancer tissue in virtually all cancer types. His research program aims to understand whether cancer cells depend on this aberrant DNA methylation profile and whether it is amenable to therapeutic intervention. He is also interested in understanding whether it is possible to modulate an anti-tumor immune response using epigenetic therapy. His work provided the scientific basis of multiple clinical trials testing this combination. Finally, He is taking advantage of this massive cancer epigenetic reprogramming to develop tissue-based and blood-based (liquid biopsy) biomarkers for sub-group classification and cancer early detection.

Joseph Ecker
Salk Institute for
Biological Studies

Joseph Ecker's group uses advanced technologies in single cell genomics and neuronal tracing to identify both the molecular signatures of individual neuronal cell types in the mammalian brain (mouse, marmoset, human) and their synaptic partners. We have developed a variety of multi-omic assays including DNA methylation, RNA-seq, chromatin accessibility and 3D structure to study single neurons across the brain. These detailed cell atlases reveal novel cellular taxonomy in the brain and elucidate spatial diversification in fine cell subtypes allowing the identification of cell type-specific DNA regulatory elements.

Emma Farley
University of California
San Diego

Emma Farley is Assistant Professor at the University of California, San Diego. Her lab uses high-throughput functional approaches in developing embryos to decipher how enhancers encode the instructions for successful development and to pinpoint enhancer mutations associated with disease. She studied Biochemistry at Oxford University and received her Ph.D. in Developmental Biology from Imperial College London. Emma worked in Mike Levine's lab at UC Berkeley and Princeton University as a postdoc, where she exploited the advantages of the model organism Ciona intestinalis (the sea squirt) for functional genomics. She developed methods to create and functionally test millions of enhancer variants in every cell of a developing embryo. Her research enabled the first high-throughput dissection of an enhancer within whole developing embryos, these studies revealed regulatory principles governing enhancer function.

John Greally
Albert Einstein College of Medicine University

John Greally is a tenured Professor in the Departments of Genetics (Chief, Division of Genomics), Medicine (Division of Hematology) and Pediatrics (Division of Genetics) at the Albert Einstein College of Medicine, where he has been on faculty since 2001. He was also the first Affiliate Member appointed at the New York Genome Center. A native of Galway, Ireland, Dr. Greally received his honours degree in Medicine from the National University of Ireland in Galway in 1988, subsequently moving to the Children's Hospital of Pittsburgh for Pediatrics residency and Yale University in 1993 for subspecialty training in Clinical Genetics. He received his higher degrees in medicine (D.Med.) and science (Ph.D.) degrees from the National University of Ireland, Galway. He was awarded his Fellowship of the American College of Medical Genetics (FACMG) in 2013. He is the Founding Director of the Center for Epigenomics at Einstein, where he holds the endowed position of Faculty Scholar for Epigenomics. He is an active clinician, with hospital privileges at Montefiore Medical Center in the Bronx, where he is an attending physician seeing patients referred for genetic problems. He is part of the NIH's CSER Consortium, developing software tools to enhance patient diagnostic success, clinical decision support and reverse phenotyping. His basic science research is focused on the use of genomics techniques to understand human disease pathogenesis, described as somatic cellular genomics, specifically looking at cellular epigenetic models of reprogramming and cell subtype compositional changes.

Myriam Hemberger
University of Calgary

Myriam Hemberger trained at the University of Freiburg and Max-Planck Institute for Molecular Genetics, Berlin, Germany, and as postdoctoral fellow at the Samuel Lunenfeld Research Institute in Toronto and at the University of Calgary, Canada. From 2004-2019, she held a Group Leader position at the Babraham Institute in Cambridge, UK, first as MRC Career Development Fellow and since 2009 on a tenured position as part of Babraham's Epigenetics Programme. As of October 2018, she joined the University of Calgary and Alberta Children's Hospital Research Institute (ACHRI) as a full professor in the Departments of Biochemistry & Molecular Biology and Medical Genetics. She is also Theme Lead of ACHRI's Genes, Development & Health research focus area. Dr. Hemberger's scientific expertise is centred on early developmental processes that underpin normal placentation and consequently a healthy reproductive outcome. In 2007 she was awarded the IFPA Award in Placentology, and in 2019 the March of Dimes and Richard B. Johnston, Jr., MD Prize in Developmental Biology for her contributions to the field. A particular focus area of her research over the past years has been on the genetic-epigenetic crosstalk in the regulation of mouse trophoblast stem cell (TSC) self-renewal and differentiation. She has been involved in the pioneering advance of generating human TSCs and has led a systematic analysis of the impact of mouse embryonic lethal gene mutations on placentation as part of a large phenotyping consortium (dmdd.org.uk). Leading on from these insights, her current work focuses on comparatively investigating transcriptional and epigenomic modifiers in mouse and human TSCs, and on establishing a molecular basis for the impact of physiological and environmental influences on placentation in normal and abnormal pregnancies.

LeAnn Howe
University of British Columbia

LeAnn Howe, PhD, is a Canadian scientist working to understand how gene expression programs are maintained. She obtained her PhD at the University of Victoria, Canada, under Professor Juan Ausió, and was one of the first to use chromatin immunoprecipitation to map histone post-translational modifications (PTMs) at active genes. Dr. Howe subsequently characterized multiple histone acetyltransferase complexes during her post-doctoral training in the laboratory of Professor Jerry Workman at the Howard Hughes Medical Institute at Pennsylvania State University, resulting in a number of seminal publications in Science and Genes & Development. In 2003, Dr. Howe returned to Canada, as an independent investigator in the Department of Biochemistry and Molecular Biology at the University of British Columbia, which consistently ranks among the 40 best universities in the world. At UBC, Dr. Howe's group has expanded our understanding of how chromatin shapes transcriptional programs with publications in journals such as Proceedings of the National Academy of Science and Nature Communications. Chief among her group's contributions is the identification and characterization of multiple histone PTM "readers".

Miguel Ramalho-Santos
University of Toronto

Miguel grew up in Coimbra, Portugal, and attended the University of Coimbra for an undergraduate degree in Biology and a Masters' degree in Cell Biology, under the supervision of Carlos Faro. He moved to the US in 1997 for his PhD at the Department of Molecular and Cellular Biology of Harvard University, where he was co-advised by Doug Melton and Andy McMahon. He received his PhD in 2002 and in 2003 moved to San Francisco to become a UCSF Fellow, an independent research position designed as an alternative to a traditional postdoc. In 2007 he became an Assistant Professor at UCSF, and he was promoted to Associate Professor in 2013. He is the recipient of a 2008 NIH New Innovator Award and a 2016 Royan International Research Award in Reproductive Genetics. In 2018 he moved to Canada and was awarded the Canada 150 Research Chair in Developmental Epigenetics. He is Senior Investigator at the Lunenfeld-Tanenbaum Research Institute, Chair in Human Development and Full Professor in the Department of Molecular Genetics, University of Toronto.

Sheila Teves
University of British Columbia

Dr. Sheila Teves is an Assistant Professor of Biochemistry and Molecular Biology at the University of British Columbia. She received her PhD at the University of Washington in Seattle, working with Dr. Steven Henikoff at the Fred Hutchinson Cancer Research center. In her graduate thesis, she addressed the questions of how aspects of the nucleosome, from structure to dynamics, impact transcription regulation, and conversely, how the transcription process causally affects nucleosome dynamics. She then joined the laboratory of Dr. Robert Tjian at the University of California, Berkeley where she received a Jane Coffin Childs Postdoctoral Fellowship to study how transcription programs are maintained and regulated during the cell cycle. In July 2018, she started her own group at the University of British Columbia, combining interdisciplinary techniques such as genomics and single molecule imaging to address basic biological questions on transcription regulation, chromatin biology, and cell identity.

Workshop: Challenges and Opportunites in Epigenetic Studies

This workshop will address current challenges and opportunities in human population epigenetic studies, including their design, sample choice, interpretation, utility and future resources in development. We will address the biological complexity of heterogeneous tissues and cells, the influence of genetic sequence variation, as well as the design of meaningful and interpretable experimental and epidemiological approaches to test mechanistic and biomarker hypotheses. Computational and analytical approaches will be discussed to address challenges and harvest opportunities to accelerate our understanding of epigenetics in health and disease.

Moderator: John Greally, Albert Einstein College of Medicine University

Kelly Bakulski
University of Michigan

Kelly M. Bakulski, Ph.D. is an Assistant Professor in the Department of Epidemiology at the University of Michigan School of Public Health. Dr. Bakulski's research goal is to understand the environmental and genetic etiologies of neurological disorders, including autism spectrum disorder and Alzheimer's disease. Dr. Bakulski incorporates population approaches and laboratory experiments to develop biomarker and cell type tools to better inform epigenetic inferences.

Andres Cardenas
University of California Berkeley

Andres Cardenas PhD, MPH is an Assistant Professor in the Division of Environmental Health Sciences and faculty member in the Center for Computational Biology at the University of California, Berkeley. Dr. Cardenas applies epidemiological and molecular approaches to evaluate the contribution of environmental exposures in the development of disease. He is currently investigating the prenatal influence of exposure to multiple metals, air pollution, endocrine disrupting compounds, diet and maternal medication use on the epigenome of newborns and children. His current research evaluates the role of environmental exposures in utero, epigenetic modifications, and their role in the developmental origins of health and disease.

Meaghan Jones
University of Manitoba

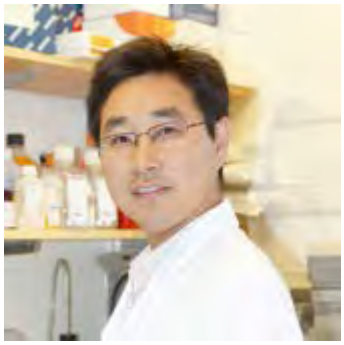
Dr. Meaghan Jones uses a combination of human population epigenetic data and controlled exposure animal models to ask whether epigenetics might serve as a long-term cellular memory of prenatal and early life environments that goes on to affect lifelong health. In particular she is interested in epigenetic marks induced by inhaled pollutants, including tobacco and cannabis smoke, and how these marks might influence health outcomes including asthma and obesity throughout the lifespan. Dr. Jones also has an interest in aging and the phenomenon of epigenetic age. She is an Assistant Professor in the Department of Biochemistry and Medical Genetics in the Rady Faculty of Health Sciences, and a Scientist at the Children's Hospital Research Institute of Manitoba.

Abhinav Nellore
Oregon Health & Science University

Abhinav Nellore is an Assistant Professor in the Computational Biology Program at Oregon Health & Science University (OHSU), where he works between the Departments of Biomedical Engineering and Surgery. His group specializes in reanalysis and harmonization of publicly available genomics datasets, facilitating their reuse for other investigators while mining them for new insights. This work has primarily focused on human RNA sequencing data. Contributions include the recount2 resource that allows investigators to interrogate expression data across over 70,000 human RNA-seq samples, a global study of splicing across Sequence Read Archive samples, and a study that examines the possible origins of putative cancer-specific splicing. Nellore's group has recently been making strides towards developing recount-methylation, an analog to recount2 for raw HM450K and EPIC methylation array data from the Gene Expression Omnibus.

Abstract Selected Speakers

Aniruddha Chatterjee
Head of Laboratory, Rutherford Discovery Fellow
University of Otago



Housheng Hansen He
Senior Scientist
Princess Margaret Cancer Centre

Gurbet Karahan
Postdoctoral Fellow
McGill University

David Labbe
Assistant Professor
McGill University

Ben Laufer
Postdoctoral Fellow
University of California (Davis)

Louis Lefebvre
Associate Professor, Department of Medical Genetics
University of British Columbia

Danny Leung
Assistant Professor
The Hong Kong University of Science and Technology

John McCarrey
Professor, Kleberg Distinguished University Chair in Cellular & Molecular Biology
The University of Texas at San Antonio

Victor Medved
Postdoctoral Fellow
Ottawa Health Research Insitute

Anna Panchenko
Canada Research Chair in Computational Biology and Biophysics
Queen's University

Alan Underhill
Associate Professor, Medicine & Dentistry-Oncology
University of Alberta

Noha Yousri
Assistant Professor of Research in Genetic Medicine
Weill Cornell Medical College, Qatar

Rapid Fire Talks

Luis Eduardo Abatti
PhD Candidate
University of Toronto

Supervisor
Jennifer Mitchell

Christopher Cafariello
MSc Candidate
Ottawa Hospital Research Institute

Supervisor
William Stanford

Lin Gao
MSc Candidate
Hong Kong University of Science and Technology

Supervisor
Danny Leung

Aurélie Huang Sung
MSc Candidate
Institut de recherches cliniques de Montréal

Supervisor
Nicole Francis

Sima Khazaei
PhD Candidate
McGill University

Supervisor
Nada Jabado

Amy Inkster
PhD Candidate
BC Children's Hospital Research Institute

Supervisor
Wendy Robinson

Rashedul Islam
PhD Candidate
University of British Columbia

Supervisor
Martin Hirst

Anne-Sophie Pépin
PhD Candidate
McGill University

Supervisor
Sarah Kimmins

Liangxi Wang
PhD Candidate
University of Toronto

Supervisor
Michael Wilson

Stanley Zhou
PhD Candidate
University of Toronto

Supervisor
Mathieu Lupien

Epigenetic Technologies

Fergus Chan
Director, Product Management
10X Genomics

Kin Kuok
Field Application Scientist
MGI Americas

Justin Lin
Scientist
Zymo Research

Anthony Schmitt
VP R&D
Arima Genomics

IHEC Workgroup Meetings

Sunday, November 17th, 2019

07:00 - 08:30	Breakfast *(Primrose)*
BREAKOUT SESSIONS	
08:30 - 10:00	Data Ecosystem / Assay Standards and Quality Control (Hawthorn) Chair: Daniel Zerbino / Martin Hirst
08:30 - 10:00	BioEthics (Bluebell) Chair: Yann Joly
10:00 - 10:30	Coffee Break
10:30 - 12:00	Integrative Analysis (Hawthorn) Chair: Guillaume Bourque
10:30 - 12:00	Communication (Bluebell) Chair: Eric Marcotte
12:00 - 13:15	Lunch (Wildrose A)
GENERAL SESSIONS	
13:15 - 13:25	Welcome and General Remarks (Wildrose BC)
	CONSORTIUM UPDATES
13:25 - 13:35	Canada (Eric Marcotte / Martin Hirst)
13:35 - 13:50	EU SYSCID / MultipleMS (Tomasz Dylag / Philip Rosenstiel / Chris Cotsapas) EU STANDS4PM (Stephan Beck)
13:50 - 13:55	Germany (Jorn Walter)
13:55 - 14:00	Hong Kong (Danny Leung)
14:00 - 14:15	Japan (Toshikazu Ushijima / Hiroyuki Sasaki)
14:15 - 14:20	Singapore (Shyam Prabhakar)
14:20 - 14:25	South Korea (TBD)
14:25 - 14:35	USA 4D Nucleome / HubMAP (Ananda Roy)
14:35 – 14:40	USA ENCODE (Mike Pazin)
14:40 - 15:00	Coffee Break
	WORKING GROUP UPDATES
15:00 - 15:10	Assay Standards and Quality Control (Martin Hirst)
15:10 - 15:20	BioEthics (Yann Joly)
15:20 - 15:30	Data Ecosystem / Assay Standards and Quality Control (Daniel Zerbino)
15:30 - 15:40	Communication (Eric Marcotte)
15:40 - 17:00	**FUTURE OF IHEC: Integrative Analysis** Chair: Martin Hirst / Guillaume Bourque

IHEC Science Days / CEEHRC Agenda

Sunday, November 17th, 2019

16:30 - 18:30	Registration *(Wildrose Prefunction)*
KEYNOTE PRESENTATION I	
18:30 - 19:30	Bing Ren (University of California San Diego) *Epigenomics of Single Cells*
19:30 - 21:30	Cocktail Reception *(Hawthorn)*

Monday, November 18th, 2019

07:00 - 07:55	Breakfast *(Wildrose)*
07:55 - 08:00	Opening Remarks Martin Hirst
Session 1: Epigenetic Programming Chair: Guillaume Bourque	
08:00 - 09:00	**KEYNOTE PRESENTATION II** John Stamatoyannopoulos (University of Washington) *Reading and programming the Epigenome*
09:00 - 09:30	Jacques Côté (Université Laval) *Recurrent chromosomal translocations that subvert The NuA4/TIP60 chromatin modifying complex*
09:30 - 09:45	Louis Lefebvre (University of British Columbia) *Evolution of imprinting via lineage-specific insertion of retroviral promoters*
09:45 - 10:15	Coffee Break
Session 2: Transcriptional Regulation Chair: Cheryl Arrowsmith	
10:15 - 10:45	LeAnn Howe (University of British Columbia) *Maintenance of Active Gene Expression States*
10:45 - 11:15	Miguel Ramalho-Santos (University of Toronto) *Epigenetic Regulation of Pluripotency*
11:15 – 11:30	Danny Leung (Hong Kong University of Science and Technology) *G9a regulates DNA methylation and chromatin looping in distinct mechanisms*
11:30 - 12:30	Lunch
12:30 - 14:30	Banff Bus Tour Sightseeing bus tour of local attractions in Banff
Session 3: Epigenetics in Disease I Chair: Carolyn Brown	
14:30 – 15:00	John Greally (Albert Einstein College of Medicine) *Somatic cellular genomics and human disease insights*

15:00 - 15:15	Aniruddha Chatterjee (University of Otago) *DNA methylation levels regulate PD-L1 expression in melanoma subtypes*
15:15 – 15:30	Noha Yousri (Weill Cornell Medicine) *Epigenetic modifications of Type 2 Diabetes in a Middle Eastern Population*
Session 3: Epigenetic Technologies Chair: Serge McGraw	
15:30 - 15:45	Fergus Chan (10x Genomics) *Deciphering epigenetic regulation with single cell ATAC-seq*
15:45 - 16:00	Justin Lin (Zymo Research) *Consolidated Technologies for Epigenetics Research*
16:00 - 16:15	Anthony Schmitt (Arima Genomics) *Accurate and cost-effective discovery of active gene regulatory interactions using Arima-HiChIP*
16:15 - 16:30	Kin Kuok (MGI Americas) *Redefine NGS with MGI: DNBSEQ™ Technology for Epigenetic Research*
16:30 - 17:00	Coffee Break
Session 5: Rapid-Fire Talks Chair: Steve Bilodeau and Steven Jones	
17:00 - 17:05	Luis Eduardo Abatti (University of Toronto, PI: Jennifer Mitchell) *Epigenetic activation of a common enhancer results in SOX2 overexpression in multiple cancer subtypes*
17:05 - 17:10	Christopher Cafariello (Ottawa Hospital Research Institute, PI: William Stanford) *The Identification of MTF2-specific Synthetic Lethal Interactions in Refractory Acute Myeloid Leukemia Using CRISPR*
17:10 – 17:15	Lin Gao (Hong Kong University of Science and Technology, PI: Danny Leung) *Delineating the Transcriptional Regulatory Roles of Retrotransposons in Human Placenta*
17:15 – 17:20	Aurélie Huang Sung (Institut de recherches cliniques de Montréal, PI: Nicole Francis) *Identification of Factors Involved in the Chromatin Binding of Polycomb Group Proteins Across the Cell Cycle*
17:20 – 17:25	Sima Khazaei (McGill University, PI: Nada Jabado) *Histone H3.3 G34W mutation in neoplastic stromal cells promotes ECM remodeling and drives tumorigenesis of giant cell tumor of bone*
17:25 – 17:30	Amy Inkster (BC Children's Hospital Research Institute, PI: Wendy Robinson) *The placenta is not an asexual organ: sex-specific placental DNA methylation signatures*
17:30 – 17:35	Rashedul Islam (University of British Columbia, PI: Martin Hirst) *Dynamic epigenetic regulation during B-cell maturation and transformation to Chronic Lymphocytic Leukemia (CLL)*
17:35 – 17:40	Anne-Sophie Pépin (McGill University, PI: Sarah Kimmins) *KDM1A mediates transgenerational metabolic disturbances in a sex-specific manner and is linked to diet-induced alteration in sperm chromatin signatures*
17:40 – 17:45	Liangxi Wang (University of Toronto, PI: Michael Wilson) *Inter-species and inter-tissue comparisons highlight the contribution of a DNA transposon to inflammatory cytokine induced NF-kB binding*
17:45 – 17:50	Stanley Zhou (University of Toronto, PI: Mathieu Lupien) *The Three-Dimensional Genome Atlas of Primary Prostate Cancer*

19:00 - 20:00	Dinner *(Wildrose)*
Poster Session 1 (Hawthorn)	
20:00 - 22:00	See Poster Assignments (page 18)

Tuesday, November 19th, 2019

07:00 - 08:00	Breakfast
Session 6: Epigenetic Regulation in Development I Chair: Marjorie Brand	
08:00 - 09:00	**KEYNOTE PRESENTATION III** Ellen Rothenberg (Caltech) *Resistance and transformation in T-cell development: gene networks against epigenetic constraint*
09:00 - 09:30	Emma Farley (University of California San Diego) *Regulatory principles governing enhancer function in development and disease*
09:30 - 09:45	John McCarrey (University of Texas at San Antonio) *Unique Epigenetic Programming Distinguishes Regenerative Spermatogonial Stem Cells in the Developing Mouse Testis*
09:45 - 10:00	Ben Laufer (University of California, Davis) *Cell-free Fetal DNA Methylation Profiles of Maternal Obesity Effects on Infant Neurodevelopment in Non-human Primates*
10:00 - 10:30	Coffee Break
Session 7: Epigenetic Regulation in Development II Chair: Sarah Kimmins	
10:30 - 11:00	Myriam Hemberger (University of Calgary) *The uterine and placental epigenome during pregnancy as a function of maternal age*
11:00 - 11:30	Shelia Teves (University of British Columbia) *Transcriptional memory and dynamics in embryonic stem cells*
11:30 - 11:45	Gurbet Karahan (McGill University) *Profound hypomethylation of the sperm DNA methylome associated with MTHFR deficiency and compatible with fertility*
11:45 - 12:00	Victor Medved (Ottawa Hospital Research Institute) *Histone H3 lysine 4 mono-methylation facilitates cell fate transitions during muscle regeneration*
12:00 - 13:00	Lunch
Workshop: Challenges and Opportunities in Epigenetic Studies Moderator: John Greally	
13:00 - 14:30	Challenges & Opportunities in Epigenetic Studies • Kelly Bakulski (University of Michigan) • Andres Cardenas (University of California Berkeley) • Meaghan Jones (University of Manitoba) • Abhinav Nellore (Oregon Health & Science University)
14:30 - 15:00	Coffee Break

Session 8: Epigenetics in Disease II Chair: Mike Pazin	
15:00 - 15:30	Judy Cho (Mount Sinai) *Single cell analyses in inflammatory bowel disease: cell fate and treatment responses*
15:30 - 16:00	Daniel De Carvalho (University of Toronto) *Epigenetic regulation of Retroelements as an Emerging Therapeutic Opportunity in Cancer*
16:00 - 16:15	Housheng Hansen He (Princess Margaret Cancer Centre) *CRISPRi screen of risk-associated cis-regulatory elements reveals 3D genome dependent causal mechanisms in prostate cancer*
16:15 - 16:30	David Labbe (McGill University) *Single-cell transcriptomes reveal a MYC-driven reprograming of the androgen receptor cistrome in prostate cancer*
18:30 - 20:00	Dinner
Poster Session 2	
20:00 - 22:00	See Poster Assignments (page 19)

Wednesday, November 20th, 2019

07:00 - 08:30	Breakfast
Session 9: Epigenetic Mechanisms Chair: Nathalie Berube	
08:30 - 09:00	Joseph Ecker (Salk Institute) *Single Cell Epigenomic Analysis of the Anatomy and Neuronal Circuitry of the Brain*
09:00 - 09:30	Julie Brind'Amour (University of British Columbia) *Impact of transcription on the establishment of the oocyte epigenome*
09:30 - 09:45	Anna Panchenko (Queen's University) *Developing Hybrid Methods to Characterize Nucleosome Structure and Dynamics with High Precision*
09:45 - 10:00	Alan Underhill (University of Alberta) *Effective protein biocontainment through phase separation*
10:00 – 10:15	Coffee Break
Session 10: CEEHRC Phase II Moderator: Martin Hirst	
10:15 - 11:15	CEEHRC Network updates
11:15 - 11:30	**Close Meeting**
11:30	Bus departures

Poster Assignments

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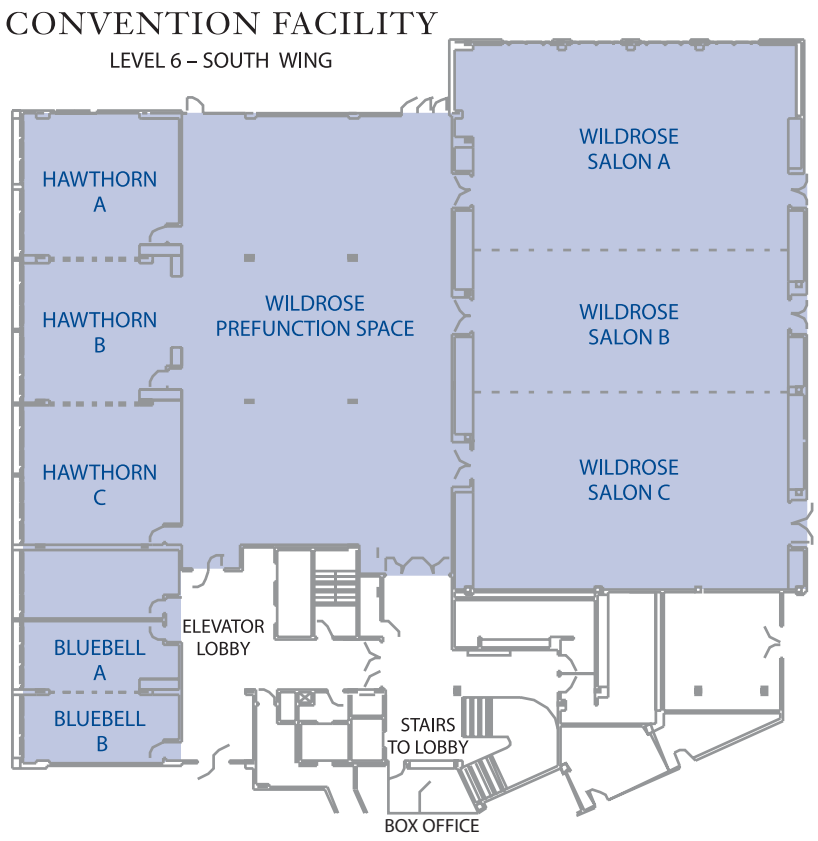

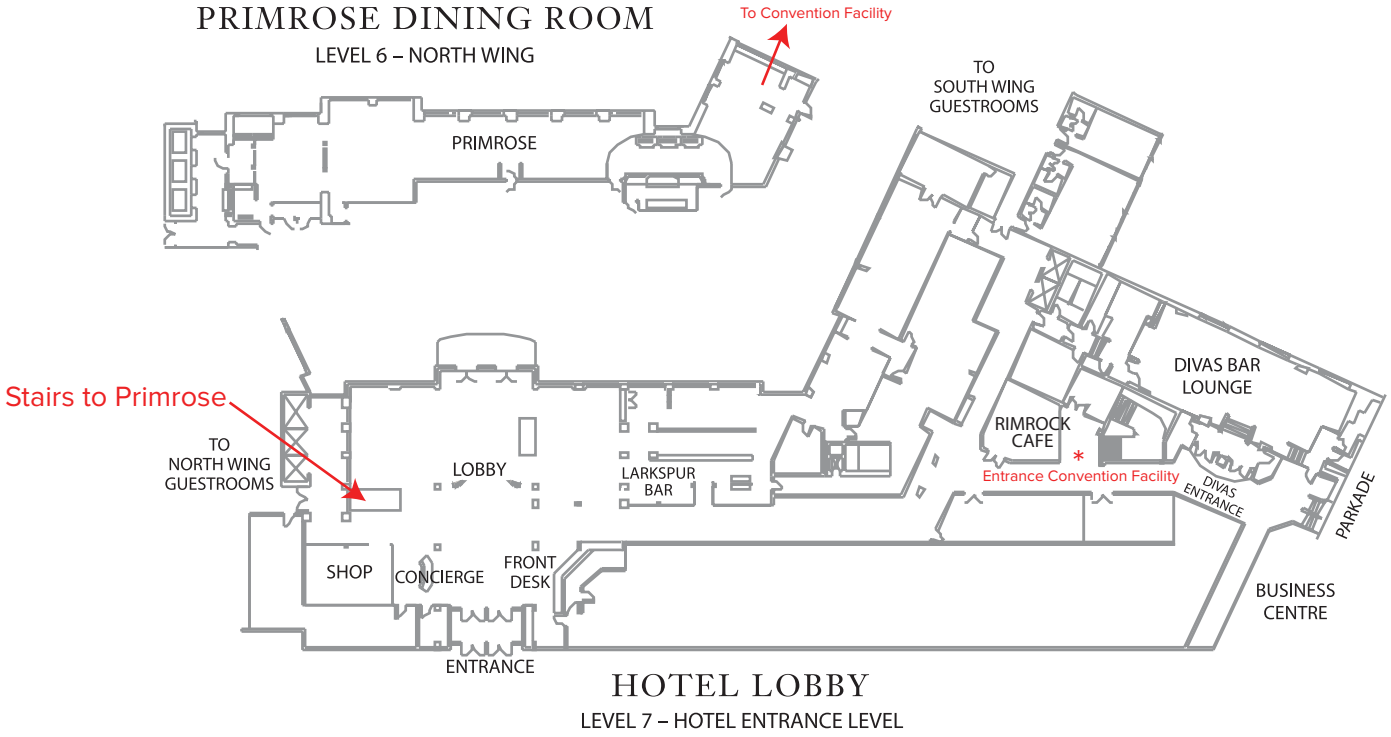

Abstracts

Word cloud showing the top keywords from this year's abstracts.
Figure was generated at https://www.wordclouds.com

EPIGENETIC ACTIVATION OF A COMMON ENHANCER RESULTS IN *SOX2* OVEREXPRESSION IN MULTIPLE CANCER SUBTYPES

Luis E. Abatti[1], Jennifer A. Mitchell[1]
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Sex-determining region Y box2 (SOX2) is a key transcription factor associated with pluripotency in embryonic and reprogrammed stem cells. In these cells, *SOX2* is essential for self-renewal, cooperating with a wide network of transcription factors to regulate pluripotency-associated genes. Transcriptional studies show that *SOX2* is highly expressed in progenitor cells, and it is normally silenced in most differentiated epithelial tissues. However, overexpression of *SOX2* has been commonly observed in at least 25 different cancer types, including breast and lung adenocarcinomas, where it has been linked to epithelial-mesenchymal transition, increased tumorigenesis and poorer patient prognosis due to its participation in the formation and maintenance of tumour-initiating cells. Tumour-initiating cells are thought to retain increased propagation potential and to quickly repopulate tumours after chemotherapy, conferring a drug-resistant phenotype. The misactivation of enhancers has been proposed as one of the main mechanisms behind aberrant gene expression in multiple diseases, including cancer. Understanding how these regulatory regions become active can help identify key upstream players driving the altered transcriptional network of cancer cells. Through the investigation of breast cancer epigenetic data, we have identified two genomic regions – SRR124 and SRR134 – with increased enhancer features, including open chromatin, H3K27ac and P300 binding in *SOX2*-overexpressing MCF-7 adenocarcinoma cells. Such enhancer features are not present in normal mammary epithelial cells, indicating that these regions may become active as cells transition to a diseased state. Homozygous deletion of both SRR124 and SRR134 regions from MCF-7 cells (SOX2-Δ124-134) using CRISPR-Cas9 reduced *SOX2* expression to nearly undetectable levels (log2FC -10.35, $p < 0.001$). Next, we investigated ATAC-seq data from TCGA cancer patients which showed that the SRR124-134 region is open in 75% of breast cancer tumours, with a significant correlation to open chromatin at the *SOX2* gene promoter in these samples ($p < 0.001$, $R^2 = 0.3736$). This indicates that the SRR124-134 region may be playing a similar role in *SOX2* overexpression in a large proportion of breast cancers compared to our MCF-7 model. Interestingly, 100% of lung adenocarcinoma patients also display an open chromatin signal at the SRR124-134 region, and this was significantly correlated to open chromatin at the *SOX2* gene promoter ($p < 0.001$, $R^2 = 0.3815$). Based on these findings, our current hypothesis is that aberrant accessibility of the SRR124-134 region causes *SOX2* overexpression in a variety of tumours, including breast and lung adenocarcinomas. Understanding the mechanisms underlying *SOX2* activation through this enhancer will illustrate the key transcription factors involved in the transcriptional changes associated with $SOX2^+$ tumour-initiating cells.

REFINING AN EPIGENETIC MOUSE MODEL OF FETAL ALCOHOL SPECTRUM DISORDER THAT INCLUDES POSTNATAL MATERNAL SEPARATION STRESS

Bonnie Alberry, Shiva M Singh
Biology Department, Western University, London, ON

Prenatal alcohol exposure results in developmental, intellectual and behavioral deficits collectively termed fetal alcohol spectrum disorders (FASD). While preventable, FASD remains a common societal burden with no cure. Children diagnosed with FASD regularly face additional environmental challenges, including maternal separation stress during ongoing neurodevelopment. The combination of prenatal alcohol exposure and early life stress in children results in a worsening of deficits. We have used a mouse model to determine if the increased developmental and behavioral deficits in such a model may be explained by associated alterations in hippocampal gene expression using RNA-Seq and weighted gene co-expression network analysis (WGCNA), with validation by qPCR. Furthermore, we use MeDIP-Seq to assess if these changes in gene expression are acquired and maintained into adulthood by altered promoter DNA methylation. While we find altered gene expression and DNA methylation profiles following prenatal ethanol exposure, postnatal maternal separation, and the combination of both treatments, the overlap between gene expression and DNA methylation is minimal. Our results suggest that an epigenetic mechanism beyond DNA methylation must be in play. Further research is needed to understand how these changes occur and persist for earlier detection and potential amelioration for children diagnosed with FASD.

EVOLUTIONARY CHARACTERIZATION OF NF-KB BINDING SITES IN MAMMALS IDENTIFIES PRINCIPAL GENE-REGULATORY MECHANISMS OF ACUTE INFLAMMATION

Azad Alizada[1,2*], Nadiya Khyzha[3,4*], Liangxi Wang[1,2], Lina Antounians[1,2], Xiaoting Chen[5], Melvin Khor[3,4], Minggao Liang[1,2], Matthew T. Weirauch[5,6,7], Alejandra Medina-Rivera[1,8], Jason E Fish[#3,4,9], Michael D Wilson[#1,2]
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The transcription factor complex NF-κB plays a central and conserved role in the inflammatory response and many disease phenotypes. NF-κB binds tens of thousands of genomic sites, but

the functional consequences are largely unknown. Here, we performed a comparative epigenomic analysis of NF-κB-chromatin interactions during an acute inflammatory response. Using primary mammalian endothelial cells treated with the pro-inflammatory cytokine TNFα, we found target gene-associated conserved orthologous (human-mouse-cow) binding of the NF-κB subunit RELA/p65 within both accessible and nucleosome-occluded chromatin. Regions with substantial RELA occupancy before TNFα stimulation were highly conserved between species and dramatically altered RELA binding, chromatin accessibility, and active histone modifications after TNFα stimulation. These RELA pre-bound sites were typically utilized by multiple cell types, fell within super-enhancers, regulated gene expression, and coincided with pleiotropic genetic variations and rare mutations associated with inflammatory diseases. We identified hundreds of conserved inflammatory super-enhancers including one at the disease associated *CCL2* locus, which was the strongest super-enhancer observed in human and mouse, and the one at the *PLK2* locus, which was ~200 kb away from the promoter. We went on to test the function of individual conserved NF-κB sites within the *CCL2* and *PLK2* loci. Our deletion experiments demonstrated that 'pre-bound' NF-κB sites within super-enhancers indeed have a major impact on gene expression through both proximal and long-range interactions during acute inflammatory responses. We also show that NF-κB binding to nucleosome-occluded sites, which has been observed but largely overlooked, is evolutionarily conserved, harbour canonical NF-κB motifs, and occurs within super-enhancers near NF-κB target genes. Overall, our evolutionary and epigenomic characterization of RELA binding modes reveals novel aspects of NF-κB-mediated gene regulation and identifies a core conserved set of NF-κB elements that likely play a principal role controlling acute inflammatory responses.

DEVELOPMENTAL TIMING OF SEX BIAS IN DNA METHYLATION LEVELS IN MOUSE LIVER

Najla Alogayil[1], Qinwei Zhuang[1], Qian Xiao[2], Jose Hector Galvez[3,4], Guillaume Bourque[1,3,4], Teruko Taketo[5,6], and Anna K Naumova[1,6,7]
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Females and males have different sex chromosome (chr.) complements and differ in many aspects of their anatomy and physiology. They also have different susceptibility to disease and response to treatment. Sexual dimorphism is driven by differences in gene regulation and associated with sex bias in autosomal DNA methylation levels in humans and mice. However, the mechanisms underlying sex biased DNA methylation remain elusive. The goal of our study was to clarify the roles of sex chr. complement vs sex phenotype in the establishment of sex-biased DNA methylation using mouse models with different combinations of sex phenotype and sex chr. complement. We examined global DNA methylation in livers from adult XX females, XY males, sex reversed XY females, and females with monosomy for the maternal X chr., XO, using whole genome bisulfite sequencing and identified 1714 autosomal sex-associated differentially methylated regions (sDMRs). Most sDMRs were dependent on the sex phenotype and a smaller proportion were associated with the sex chr. complement (Zhuang et al.).
In principle, the impact of sex phenotype on methylation may be mediated by gonadal sex hormones. Indeed, an effect of testosterone on DNA methylation has been reported. In mice, fetal testes start producing testosterone by embryonic day (E) 13.5. There are three peaks of testosterone production in the life of a male mouse: the first occurs prenatally around E15.5, the second – soon after birth and the third and largest peak is at puberty (6 weeks of age). We tested the hypothesis that testosterone production caused bias in methylation in the mouse liver and that sDMRs were present as early as E14.5, i.e. soon after the onset of testosterone production by fetal testes.
Livers were collected at three different developmental stages: E14.5, prepubescent (4-week old), and adult (2-month old). DNA methylation levels at several previously validated autosomal sDMRs were determined using targeted pyrosequencing methylation assays. Our data for certain sex-phenotype dependent sDMRs show high methylation levels in fetal livers with no significant differences between females and males and a gradual decline in methylation along with an increase in the sex bias in methylation with age. This suggests that testosterone production by fetal and postnatal testes is not sufficient to cause sex bias in methylation and other factors must be involved. The investigation of the developmental timing of sex bias in DNA methylation provides insight into the mechanisms involved and serves as a starting point for further research.

INHIBITION OF CLASS I HISTONE DEACETYLASES (HDACS) AS A THERAPEUTIC STRATEGY FOR TARGETING THE BRAIN TUMOUR STEM CELL (BTSC) POPULATION OF GBM

Bahia K. Ravinder, Luchman H. Artee, Weiss Samuel
Arnie Charbonneau Cancer Institute, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Alberta, Canada.

Glioblastoma multiforme (GBM) is the most aggressive adult primary brain tumour with a median survival of about 15 months. Resistance to conventional therapies has been proposed to be due to the presence of GBM cells with stem cell-like properties known as brain tumour stem cells (BTSCs). The stemness characteristics of these cells may be attributed to epigenetic alterations that endow them with enhanced self-renewal capabilities. Among these epigenetic factors, histone deacetylases (HDACs) have been shown to play crucial roles in normal and cancer stem cell functions. There are four different classes of HDACs which are known for their role in gene silencing by deacetylating the lysine residues of histones, H3 and H4. Studies have shown that class I HDACs (HDAC1 and 2) are essential for maintaining the pluripotent state of embryonic stem cells by regulating the core stem cell genes. Conversely, differential expression of HDAC1 and 2 is required for regulating the lineage specific genes during neuro-glial development. Any dysregulation of either expression or enzymatic activity of these

different classes of HDACs can manifest as pathological events such as neurological disorders and several human cancers including GBM. Since class I and II HDACs are often dysregulated in malignant brain tumour, GBM, their functional relevance to the growth and stemness characteristics of BTSC population is poorly understood. For this study, we first determined the protein expression of class I HDACs in BTSC lines along with normal human fetal astrocytes and astrocytes derived from human induced pluripotent stem cells (hiPSCs). Interestingly, class I HDACs, especially HDAC1, 2 and 3 were highly expressed in BTSCs. We hypothesized that class I HDACs maintain BTSCs in a self-renewing stem cell state. We propose that inhibition of class I HDACs will induce changes in chromatin architecture of BTSCs, which in turn, will result in changes in cellular properties such as cell growth and self-renewal. To test this hypothesis, we screened both pan (panobinostat, pracinostat) and class I specific HDAC inhibitors (4SC202, mocetinostat and romidepsin). We found that HDAC1 and 2 specific inhibitor, romidepsin, is sufficient for reducing BTSCs growth at picomolar concentrations. Furthermore, treatment with romidepsin also reduced sphere forming capacity, a key feature of BTSCs. An on-target increase of acetylation of lysine residues of H3 and H4 was observed following treatment with romidepsin. These data suggest that class I HDACs, especially HDAC1 and 2, may be essential for maintaining viability and self-renewal in BTSCs. Further studies will examine underlying epigenetic mechanisms regulated by class I HDACs in BTSCs and the preclinical implications of targeting class I HDAC inhibitors with the use of orthotopic xenograft models.

IDENTIFICATION OF ELEMENTS WHICH CONTRIBUTE TO VARIABLE ESCAPE FROM X-CHROMOSOME INACTIVATION

Bradley Balaton and Carolyn Brown
The 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 allows the identification of epigenetic or genetic marks which contribute to escape from XCI.

To make a comprehensive list of variably escaping genes, we aggregated XCI status calls from multiple studies. Using whole genome-seq and RNA-seq data from the Center for Epigenome Mapping Technologies (CEMT), the allelic expression ratio at heterozygous SNPs was calculated in order to determine the XCI status of genes. For the majority of samples, these XCI status calls correlated well with previous XCI calls and whole-genome bisulfite sequencing data. Currently, we are examining matched histone modification data to determine how histone modifications differ across XCI status and whether they can be predictive of a gene's XCI status.

To identify the genetic underpinnings to variable escape, we compared linkage of polymorphisms with variation in XCI status but were unable to find any significant results due to limited sample size and so we are seeking access to 3000 samples from The Cancer Genome Atlas. Any polymorphisms found to significantly correlate with XCI status will be followed up in the CEMT data and independent cell culture samples. Polymorphisms which strongly correlate with a gene's XCI status may be causative of differences in XCI status across samples. Causation will be tested by using genetic engineering to change which allele is present in a cell line and see if it changes the gene's XCI status.

Another approach to identify DNA elements is to determine the ancestral XCI status of genes and identify conserved elements. We are thus data-mining bisulfite sequencing datasets in other mammalian species in order to identify the XCI status of genes. We have XCI statuses for genes in mouse, cow, sheep and horse. Sheep has the largest percent of genes escaping (22%) while mouse has the lowest (4%) and out of 59 genes with data across all species, 1 consistently escaped and 24 were consistently subject to XCI. Conservation of candidate control elements will be examined for these genes.

Overall, our goal is to identify the factors which decide which genes will escape from XCI and which genes will be subject to XCI. Genes that escape from XCI generally show a sex-difference in their expression and are contributors to sex differences in disease. In addition, genes that variably escape XCI can also contribute to inter-female differences.

VARIANCE-STABILIZED UNITS FOR SEQUENCING-BASED GENOMIC SIGNALS

Faezeh Bayat, Maxwell Libbrecht
Department of Computing Science, Simon Fraser University, Burnaby BC, Canada

Sequencing-based genomic signals such as ChIP-seq are widely used to measure many types of genomic biochemical activity, such transcription factor binding, chromatin accessibility and histone modification. The processing pipeline for these assays usually outputs a real-valued signal for every position in the genome that measures the strength of activity at that position. This signal is used in downstream applications such as visualization and chromatin state annotation. There are several representations of signal strength at a given that are currently used, including the raw read count, the fold enrichment over control, and log p-value of enrichment relative to control.

However, these representations lack the property of variance stabilization. That is, a difference between 100 and 200 reads usually has a very different statistical importance from a difference between 1,100 and 1,200 reads. Here we propose SDPM, variance-stabilized units for sequencing-based genomic signals.

SDPM are variance-stabilized, in contrast to existing units. To measure the fit to the empirical mean-variance relationship, we evaluated the log likelihood of each transformation under a max-entropy model. We found that existing units have a poor log likelihood, indicating a non-uniform variance, while SDPM has the best log likelihood.

Variance-stabilized units identify differences between cell types. We found that when genomic signals are represented in

SDPM, differences in the signal between two cell types is predictive of a functional change between the cell types. To evaluate the quality of this predictiveness, for a given pair of cell types, we propose the differential expression score, which measures the correlation of the difference in signal at a gene's promoter with the difference in that gene's expression. A high correlation indicates that we expect that units without stable variance will have low correlation because differences in signal will be overwhelmed by high-variance positions. We found that SDPM had a higher average differential expression score than the other methods we tried.

SDPM have linear relationships with other measurements. We found that when genomic signals are represented in SDPM, they have linear relationships with other data sets. To evaluate this property, we calculated the Pearson correlation between the SDPM signal at a gene's promoter and that gene's expression. Pearson correlation measures linear relationships, so the strength of Pearson correlation indicates the linearity of the relationship. We found that SDPM had a high Pearson correlation with gene expression---an average of 0.31 across the nine cell types we tested.

SDPM units will eliminate the need for downstream methods to implement complex mean-variance relationship models and will enable genomic signals to be easily understood by eye.

EPIGENETIC ACTIVATION OF ONCOGENES IN RAT HEPATOCELLULAR CARCINOMA TRIGGERED BY CHOLINE-DEFICIENT L-AMINO ACID-DEFINED (CDAA) DIET

Megan Beetch[1], Katarzyna Lubecka[2], Abigail Durkes[3], Lucinda Kurzava[4], Nadia Atallah[4], Nathaniel Villanueva[5], Tao Huan[5], Aline de Conti[6], Igor Pogribny[6], Barbara Stefanska[1]
[1]Food, Nutrition and Health, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada; [2]Department of Biomedical Chemistry, Medical University of Lodz, Lodz, Poland; [3]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA; [4]Purdue Center for Cancer Research, Purdue University, West Lafayette, IN, USA; [5]Department of Chemistry, University of British Columbia, Vancouver, BC, Canada; [6]Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, Arkansas, USA

Activation of oncogenic signaling pathways drives cancer. Recent evidence indicates that several oncogenes are activated via epigenetic mechanisms in cancer, but limited studies have used in vivo models to evaluate changes in DNA methylation patterns related to oncogenes. We aim to provide novel mechanistic insights into regulation of oncogenic signaling in an in vivo model of hepatocellular carcinoma (HCC) by first characterizing gene expression changes in healthy versus HCC livers and exploring associated DNA methylation changes.

In the present study, rats were fed a methyl donor-deficient diet (choline-deficient L-amino acid-defined diet; CDAA) for 52 weeks to induce HCC or a methyl donor-sufficient diet (choline-sufficient L-amino acid-defined diet; CSAA, healthy control group) (n=6 per group). At the end of 52 weeks, analyses of liver nodules and histopathological features were performed followed by thorough investigation of altered gene expression in CDAA livers compared to healthy livers using RNA sequencing. Further analyses of DNA methylation-associated features (SAM/SAH, one carbon metabolism, Dnmt/Tet expression) were investigated, and loci-specific DNA methylation patterns were delineated using pyrosequencing.

We confirmed fully developed HCC tumors in all rats on CDAA diet at the termination point of 52 weeks. Analysis of RNA sequencing data revealed massive disruption of metabolism-related pathways in HCC livers of CDAA rats. Most notably, one carbon metabolism and lipid distribution pathways were altered, likely contributing to the phenotypic changes observed in the liver upon CDAA diet. Specifically, apolipoproteins as well as choline- (Bhmt), folate- (Aldh1l1), and methylation reaction-related (Gnmt, Dnmt3b) genes were downregulated, while lipid packaging (Vldlr, Lpl) and glutathione-related genes (Gstp1, Gpx2) were upregulated. Furthermore, we identified genes from several oncogenic signaling pathways significantly upregulated in HCC tumors compared to healthy liver tissue, including Notch, Wnt, Hedgehog, Mapk, and Mmp. Upstream players in these pathways that were highly overexpressed in CDAA-triggered liver tumors (e.g. Jag1 from Notch, Wnt4 from Wnt, and Smo from Hedgehog) were selected for DNA methylation analysis. DNA methylation alterations within the promoter region of each candidate are being assessed by pyrosequencing. Understanding the epigenetic regulation of candidate oncogenes could have immense utility in defining therapeutic targets for HCC.

NEW HIGH-THROUGHPUT METHODS FOR THE EPIGENOMIC CHARACTERIZATION OF HUMAN DISEASE STATES

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Methods for profiling epigenomic dynamics have increased in popularity as we continue to discover new ties between chromatin structure and mechanisms of human disease. As these assays have become common practice, we have become limited by the throughput at which they can be performed and by the interpretation of combined complex datasets. Here we introduce a new platform for high-throughput epigenomics, the basis of which comes through PIXUL, a novel 96-well sonication device. PIXUL can be operated to independently deliver distinct sonication conditions to as many as twelve different sets of 8 wells within a single run, allowing users to sonicate several sample types or experiment with different conditions simultaneously. This platform has opened the door for new possibilities in concurrently analyzing the epigenomes of multiple sample types or disease states, specifically using our new High-Throughput ChIP method. Finally, we introduce Primary TAM-ChIP, a method using our specific AbFlex recombinant antibodies conjugated to a Tn5 transposome complex, to combine chromatin IP and library generation into one simple method. Primary TAM-ChIP also allows for the multiplexing of several antibodies within the same tube, further reducing the material required for a single reaction.

TROPHOBLAST STEM CELLS AND GENOMIC IMPRINTING

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Genomic Imprinting is the monoallelic expression of genes in a parent-of-origin dependent manner. Many imprinted genes are involved in placentation and embryonic growth regulation, but the placenta is a difficult tissue for NGS studies due to its heterogenous cellular composition and contamination with maternal decidua and blood. In fact, many putative maternally-expressed imprinted genes discovered in the placenta were subsequently shown to be expressed in contaminating maternal decidual cells instead. Trophoblast Stem Cells (TSCs) are an immortal cell type derived from the blastocyst and capable of giving rise to the different trophoblast lineages of the developing placenta upon differentiation. TSCs offer an attractive alternative to whole placenta for NGS studies as they represent a homogenous population with no contaminating maternal tissue. To obtain a genome-wide view of allelic usage across the mouse genome in trophoblast, we established reciprocal mouse F1 TSCs through crosses of C57BL/6 and CAST/EiJ mice and obtained a panel of lines for further analysis. Through RNA-sequencing, we documented monoallelic expression at a subset of genes in some of the lines analyzed, including known imprinted genes. These genes could be further classified into potential stochastic imprints, strain-specific imprints, and random monoallelic expression. Subsequent analysis of these lines by ChIP-seq suggests possible epigenetic mechanisms underlying these phenomena at some of these genes. We also established TSC lines carrying a mutation at an imprinted gene, *Ascl2*, which is critical for placental development, likely via the establishment of spongiotrophoblast precursors. Through RNA-seq analysis of these lines, we explored potential targets and pathways ASCL2 might be regulating during placentation. Altogether, the analysis of these diverse TSC lines highlights the strengths of this *in vitro* model system as an alternative to *in vivo* placental studies.

INTEGRATIVE ANALYSIS OF 10,000 EPIGENOMIC MAPS ACROSS 800 SAMPLES FOR REGULATORY GENOMICS AND DISEASE DISSECTION

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The vast majority of genome-wide association study (GWAS) results remain unexplained, owing to the still incomplete annotation of gene-regulatory elements and the tissues where they act. To overcome this challenge, we present EpiMap, a compendium of 823 reference epigenomes across 18 uniformly processed and computationally completed epigenomic assays. We define chromatin states, high-resolution enhancer annotations, their activity patterns, modules of common activity, upstream regulators, and downstream target gene functions. We use EpiMap to annotate 30,247 genetic variants associated with 534 traits, to recognize principal and partner tissues underlying each trait, to recognize trait-tissue, tissue-tissue and trait-trait relationships, and to partition multifactorial and polyfactorial traits into their tissue-specific contributing factors. Our results demonstrate the importance of dense, rich, and high-resolution epigenomic annotations for GWAS dissection, and yield numerous new insights for understanding the molecular basis of complex disease.

ROLE OF DNMT3B IN ACUTE MYELOID LEUKEMIA

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We have previously identified a 17-gene leukemia stem cell signature (LSC17) that strongly associates with clinical outcomes and predicts treatment response in acute myeloid leukemia (AML). The top weighted gene in this LSC17 score, *DNMT3B*, is a major regulator of DNA methylation; a central process involved in AML pathophysiology.
DNMT3B's role is highlighted in ICF1 syndrome where patients show a massive deregulation of methylation associated with chromosomal instability.
Growing evidence also suggests that RNA splicing plays a major role in the early steps of leukemogenesis. DNMT3B gene is reported to have over 30 splicing forms, including catalytically active and catalytically inactive forms. It has been shown that catalytically inactive DNMT3B variants can work as a rheostat to modulate DNMT3B activity in HEK 293T cells. Thus, any subtle changes in the levels of active versus inactive DNMT3B variants can broadly modulate DNA methylation.
However, in AML, which of the DNMT3B splice variant(s) are expressed, and whether these contribute to AML development or chromosomal instability has never been studied.
Interestingly, we demonstrate using 3 independent cohorts (579 AML patients) with 3 independent techniques (RNA-Seq, proteomic, nanopore) that a short-spliced form of DNMT3B is the one that is upregulated in AML fractions enriched in LSC, but also upregulated between 12 paired diagnosis-relapse primary AML samples. This form is catalytically inactive.
Given the role of DNMT3B in controlling a proper chromosome segregation, this led us to question whether the overexpression of the catalytically inactive isoform of DNMT3B in AML correlates with deregulation of methylation and ultimately chromosomal instability. Indeed, we found across 120 primary AML patient samples, that its expression correlates with reduced methylation levels and subsequently with chromosomal anomalies, looking through 3 independent cohorts (579 primary AML patients).
Moreover, analysis of two independent AML cohorts (n=260 patients) shows that the expression of this DNMT3B short form synergizes with the presence of TP53 mutations to increase chromosomal instability. We then validated both *in vitro* and *in vivo*

a synergy between TP53KO and this DNMT3B variant, to increase proliferation in multiple models including a TP53 mutated AML cell line (HL-60), a human-derived lineage negative cord blood retrovirally transduced with TLS-ERG and in three independent TP53KO primary AML cell models generated with CRISPR/Cas9. Finally, we showed that expression of this DNMT3B form is associated with reduced overall survival as well as chemoresistance, in 120 AML patient samples.
Because DNMT3B is not only overexpressed in AML, but also in a broad range of cancers, this study suggests a broader role of DNMT3B deregulations beyond AML. Overall, this work may also reveal novel therapeutic approaches through pharmacologic manipulation of methylation to prevent chromosomal instability and its high adaptation/resistance to chemotherapies.

ANALYSES OF EPIGENETIC MARKS AND MECHANISMS IN DISEASE: YOUR GUIDE TO A SUCCESSFUL CUT&RUN ASSAY.

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Like the chromatin immunoprecipitation (ChIP) assay, Cleavage Under Targets & Release Using Nuclease (CUT&RUN) is a powerful and versatile technique used for probing protein-DNA interactions within the natural chromatin context of the cell. The CUT&RUN assay can be combined with downstream qPCR or NG-seq to analyze histone modifications and binding of transcription factors, DNA replication factors, or DNA repair proteins at specific target genes or across the entire genome.
CUT&RUN provides a rapid, robust, and true low cell number assay for detection of protein-DNA interactions in the cell. Unlike the ChIP assay, CUT&RUN is free from formaldehyde cross-linking, chromatin fragmentation, and immunoprecipitation, making it a much faster and more efficient method for enriching protein-DNA interactions and identifying target genes. CUT&RUN can be performed in as little as one to two days, from live cells to purified DNA, and has been shown to work with as few as 500-1000 cells per assay (1,2). Instead of fragmenting all of the cellular chromatin as done in ChIP, CUT&RUN utilizes an antibody-targeted digestion of chromatin, resulting in much lower background signal than seen in the ChIP assay. As a result, CUT&RUN requires only 1/10th the sequencing depth that is required for ChIP-seq assays. Finally, the inclusion of simple spike-in control DNA allows for accurate quantification and normalization of target-protein binding between samples. This provides for effective normalization of signal between samples and between experiments.
I will discuss the basics of the CUT&RUN assay and important factors to consider when setting up your experiment. In addition, I will introduce Cell Signaling Technology's new CUT&RUN Assay Kit and CUT&RUN pAG-MNase enzyme, and present data showing the versatility of this assay for mapping various histone modifications, transcription factor, and transcription cofactor binding across multiple cell types.

A NOVEL ENZYMATIC APPROACH TO STUDY CELL-FREE DNA METHYLATION: TOWARDS THE IDENTIFICATION OF BIOMARKERS IN EPILEPSY

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Epilepsy affects more than 50 million people worldwide and mesial temporal lobe epilepsy (MTLE) is the most frequent type in adult patients. The misdiagnosis of epilepsy occurs in around 25% of patients, and approximately 30% of patients with MTLE do not respond to treatment with antiepileptic drugs and could benefit from epilepsy surgery. Therefore, the search for biomarkers to assist in the diagnosis of epilepsy as well as for identifying drug-resistant patients would be of great importance. Methylated cell-free DNA (met-cfDNA) has recently emerged as a potential biomarker since it can be analyzed and quantified noninvasively. Furthermore, met-cfDNA have already been described as a diagnostic biomarker in several diseases. Our work aims to determine if there are epigenetic signatures present in cfDNA that may help improve the diagnosis and the treatment management of patients with MTLE. This is an ongoing project, and it has been divided into different phases: i) the isolation and quantification of cfDNA present in plasma of patients with MTLE and controls; ii) determination of the methylation pattern of cfDNA using whole-genome methylation analysis of cfDNA; iii) identification of differentially methylated regions (DMRs) in patients with MTLE in comparison with controls, and in drug-resistant in comparison with drug-responsive patients; iv) the construction of customized panels containing the identified DMRs to be tested in a second and larger cohort of patients with MTLE. The first cohort of patients with MTLE is composed of 10 patients with drug-resistant MTLE, 10 with drug-responsive, and 10 control subjects. The independent cohort has 100 patients with MTLE and 100 controls. To date, we have completed the recruitment of the two cohorts and the isolation of the plasma cfDNA in all samples was accomplished. Our preliminary results indicate that there is a modest increase in the total concentration (ng) of cfDNA in the plasma of patients with drug-refractory MTLE (29.4 ± 4.9) in comparison with drug-responsive MTLE (25.1 ± 4.1), and healthy controls (25.6 ± 4.2). Moreover, we test a new method, enzyme-based approach (EM-seq) without bisulfite for the methylation study since enzymatic conversion is highly effective and minimizes DNA damage, allowing for superior detection of 5mC and 5hmC from fewer sequencing reads. Our results using EM-seq indicates a global cfDNA hypomethylation profile in patients with MTLE. Interestingly, under both conditions, most unmethylated sites were not seen in CpG contexts, but in CHH and to a lesser extent in CHG contexts. Although preliminary, our results are very encouraging, showing good potential for the use of EM-seq in the identification of cfDNA biomarkers in epilepsy.

EPISHARE: A PLATFORM TO SECURELY SHARE EPIGENOMIC CONTROLLED ACCESS DATA

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EpiShare is an open science project involving the International Human Epigenome Consortium (IHEC) that aims to develop tools and APIs to increase accessibility of epigenomic data. It does so by using and contributing to standards established by the Global Alliance for Genomics and Health (GA4GH), such as the rnaget API specification to store and query transcriptomic data in formats that are compatible with other large-scale projects (e.g. Human Cell Atlas).
It will offer methods to easily discover available epigenomes, and to launch multi-omics analyses on restricted access datasets at their storage location when possible, or to otherwise bring them to selected compute services. This will be done by developing an infrastructure of nodes to securely store and make epigenomic (meta)data available. Analysis tools will be packaged as containers that can be deployed and executed at remote locations. Our proposal contrasts with the current approaches that require researchers to locally download and clean up data and metadata coming from many epigenomic projects, which is both time-consuming and compute resources inefficient.
To demonstrate the feasibility of such a system, we implemented Varwig, a prototype portal that enables researchers to explore the interaction between genomic variants and epigenetic features, such as histone tail modifications. Building on resources we have implemented to share publicly available processed data, such as the IHEC Data Portal (epigenomesportal.ca/ihec), the Varwig tool stores genomic and epigenomic data from the BLUEPRINT EpiVar project, and allows users to visualize the effect of variants on various epigenomic features for a chosen genomic range. Sensitive data, such as per-individual genotypes, are never exposed to the portal users. Graphs and annotation tracks per genotype and per experiment are generated on demand, and visualizable on the UCSC Genome Browser, allowing users to assess differences across those genotypes.
The EpiShare prototype and resources are hosted by GenAP (genap.ca) and funded by Genome Canada, and leverage resources from the IHEC Data Portal, an online resource funded under the CEEHRC, by the CIHR and Genome Quebec, with additional support from Genome Canada. The computing and networking infrastructure, and part of the software development, are provided by Compute Canada and CANARIE.

THE IDENTIFICATION OF MTF2-SPECIFIC SYNTHETIC LETHAL INTERACTIONS IN REFRACTORY ACUTE MYELOID LEUKEMIA USING CRISPR

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Acute myeloid leukemia (AML) is characterized by expansion of abnormally differentiated, hyper-proliferative myeloid cells in bone-marrow and blood. It is the most common form of adult leukemia in Canada with a disappointing overall survival rate below 40%. Dismal outlook among patients is the result of vast heterogeneity in the molecular etiology of AML; including abnormal cytogenetics, gain and loss of function mutations, and altered epigenetic landscapes rendering a single treatment regimen unlikely to suit all patients. Despite recent advances in risk stratification systems to better manage the disease, robust biomarkers identifying chemo-resistance are still lacking. Our laboratory has previously demonstrated that loss of epigenetic repression by the polycomb repressive complex 2 (PRC2), which is mediated by complex member metal response element binding transcription factor 2 (MTF2), drives chemo-resistance in AML. Our systems biology analyses revealed that MDM2, a negative regulator of p53, is a direct target of MTF2/PRC2. Furthermore, we demonstrated both *in vitro* and *in vivo* that MTF2-deficient refractory AML cells treated with a MDM2 inhibitor in combination with standard induction drugs were able to undergo p53-mediated apoptosis. Since approximately 8% of AML patients express mutant p53, it is unlikely that this strategy will work for all chemoresistant patients. Furthermore, at relapse patients develop resistance to drugs used previously in treatment. In this study, to identify alternative therapeutic options, a genome-scale CRISPR Knock-out (GeCKO) synthetic lethal screen was performed in matched MTF2-deficient and rescued THP-1 cells.
Careful analysis of screening data has revealed 104 highly significant (FDR>0.05) MTF2-specific synthetic lethal interactions. To develop a validation strategy, commercially available small molecule inhibitors targeting druggable MTF2-specific synthetic lethal interactions were tested using a standard apoptosis assay in matched MTF2-deficient and rescued THP-1 cells. Among eight small molecule inhibitors tested, two were effective. In the near future, validation of non-druggable targets will involve lentiviral delivery of shRNAs in patient-derived bone-marrow aspirates which are more representative of the true clonal and hierarchical nature of AML. Eventually, *in vitro* validated targets will be validated *in vivo* using a patient derived xenograft (PDX) preclinical animal model of AML using immunocompromised NOD *scid* gamma (NSG) mice.

A STATISTICAL FRAMEWORK TO DEFINE AND COMPARE REFERENCE EPIGENOMES

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As part of the International Human Epigenome Consortium (IHEC) project, a large collection of reference human epigenomes is being generated spanning a comprehensive set of tissue and cell types relevant to health and disease. IHEC epigenome maps consist of a core set of six histone modifications defined by ChIP-seq, whole genome DNA methylation (WGBS) and gene expression (RNA-seq). Quality standards were implemented to define minimum criteria and core QC metrics for each assay. One crucial characteristic of this big data resource lies in the fact that multiple samples exist for the same tissue or cell type. These replicates provide a basis to assess multiple distinct epigenomes and ultimately serve to define a reference epigenome with cell-type specific features. Here we present a statistical framework for the integrative analysis of human epigenomes and a new approach for discovering cell type-specific regulatory regions.
For the purpose of scalability, flexibility and parallelization, the data are stored and analyzed in a PostgreSQL relational database allowing for fast and complex queries. For ChIP-seq data, RPKM serves as our continuous measure of signal strength at a single sample level while MACS2 significantly enriched regions ascertain the presence or absence of the histone modification of interest. Replicates within a given tissue or cell type are combined to generate group-level tables containing a maximum-likelihood estimate and a confidence interval of the histone mark probability across the genome in 50 base-pair bins. Differentially marked regions between two groups (e.g. malignant versus normal) are detected using exact tests of proportions. Similarly, shared and differentially marked regions between multiple groups are identified using Pearson's chi-squared test with Yate's continuity correction.
Our methodology reveals a picture of the entire epigenome, identifying regions that are consistently marked irrespective of cell-type, as well as highly variable regions that may contribute to cell differentiation or tumorigenesis. We employed a leave-one-out cross validation to prove that these differential regions can be used as features to classify all nine of our sample types with high accuracy. Our results were further validated using an external test set of blood samples obtained from the Blueprint project showing that our classifier is accurate independently of the sequencing centre from which the data originates.
In conclusion unlike ChromHMM software that relies on multiple histone marks to identify chromatin states, our approach has the advantage of requiring just a single histone mark and chromosome. This new statistical approach based on the computation of epigenetic probabilistic states highlights the breadth of the IHEC high resolution epigenomic maps and provides insights into the definition of a reference human epigenome that will serve as a guide in future epigenomic studies of human diseases.

SPERM DNA METHYLOME ABNORMALITIES OCCUR BOTH PRE- AND POST-TREATMENT IN MEN WITH HODGKIN'S DISEASE AND TESTICULAR CANCER

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Combination chemotherapy has become standard for the treatment of many cancers and has contributed to increased survival from Hodgkin's disease and testicular cancer. However, questions concerning the quality of sperm after treatment have arisen. Indeed, studies have shown that DNA damage and aneuploidy persist up to 2 years following treatment. As well, previous animal studies have shown that anticancer drugs can alter the sperm epigenome. Our objectives were to ascertain the impact of Hodgkin's disease and testicular cancer (pre-treatment) and their treatment with combination chemotherapy (post-treatment) on sperm DNA methylation.
Semen samples were collected from community controls (CC, n=7) and from men undergoing treatment for Hodgkin's disease (HD, n=7) or testicular cancer (TC, n=6). Samples were collected before the start of chemotherapy (baseline) and serial samples taken at 6-month intervals post-treatment. Sperm DNA methylation was assessed with Human Methylation 450K BeadChip arrays (450k array). Bisulfite pyrosequencing was used to validate altered methylation.
Several probes of the 450k array were found within imprint control regions of imprinted genes, which demonstrated no altered methylation for any group. Hierarchal clustering of probes analyzed at baseline (385,553 probes) did not result in clustering of groups; however, significant probes discovered following 3-group ANOVA (11,525 probes), did distinguish TC (and to a lesser extent HD) patients, from CC. Post-hoc Tukey's test discovered differential methylation between HD (3,047 probes) and TC (7,760 probes) patients when compared to CC; probes found within 1kb were merged to discover regions of differential methylation. For TC patients, the promoter region of *GDF2*, contained 6 probes that were differentially methylated. Bisulfite pyrosequencing validated the altered methylation of these sites, as well as additional CpGs not assayed by the 450k array. In order to assess alterations in DNA methylation over time/post-chemotherapy, serial samples from individual patients were compared with their baseline sample. With a cut-off of beta-value >0.2 (i.e. 20% methylation change), CC individuals demonstrated on average <200 sites with altered methylation at 6, 12 and 18 months. Compared to CC individuals, half of the men undergoing treatment demonstrated 20-fold higher alterations in DNA methylation at 6 months post-chemotherapy. While most patients showed similar levels to CC at later time points, one HD patient still had altered sperm DNA methylation at 12 and 24 months.
From these results, distinct sperm DNA methylation signatures are present pre-treatment in men with HD and TC and may help explain increases in birth defects seen in large clinical studies.

Epigenetic defects in sperm of some cancer survivors are present even up to two years post-treatment. Abnormalities in the sperm epigenome both pre- and post-chemotherapy may have detrimental effects on future reproductive health. (Supported by CIHR).

EXPRESSION OF HUMAN ONCOGENIC MUTANT HISTONES H3.3 IN DROSOPHILA MELANOGASTER DISRUPTS DEVELOPMENT AND COMPROMISES TISSUE-SPECIFIC TRANSCRIPTIONAL REGULATION

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H3.3K27M and H3.3K36M mutations in histone H3.3 are frequently found in gliomas, chondroblastomas and a subset of head and neck squamous cell carcinomas. To help elucidate some of the mechanisms that are important to the development of these cancers, we generated a transgenic Drosophila model expressing these two mutations.
Expression of either H3K27M or H3K36M, but not wild-type H3.3, in many Drosophila tissues is lethal, and expression in eye discs induces rough and small eyes. H3K27M expression reduces H3K27me3 but increases levels of H3K36me2, while the reverse is observed upon expression of H3K36M. Notably, the eye phenotypes of H3K27M and H3K36M respectively are suppressed by co-expressing shRNA targeting the H3K27 methyltransferase E(z) and the H3K36 methyltransferase ash1, and normal H3K36me2 and H3K27me3 levels are restored. Expression of H3.3K27M or H3.3K36M results in downregulation of genes involved in eye development, and also lead with H3.3K27M to massive upregulation of genes in the piwi-interacting RNA (piRNA), normally restricted to germline cells.
H3.3K27M and H3.3K36M mutations lead to decreased deposition and spread of the chromatin marks they affect, but also promote the spread of antagonistic chromatin marks which results in inappropriate activation of germ-cell specific genes, defective differentiation, and tumorigenesis.

INTEGRATIVE GENOMICS IDENTIFY OLIG2 AS A REGULATORY FACTOR IN SHH SUBGROUP MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most malignant brain tumour in children. Survival outcomes of MB are poor, and relapse is common. Our lab has published evidence of a cellular hierarchy in a mouse model of the sonic hedgehog (SHH) subgroup medulloblastoma, characterized by hyper-activated SHH pathway signalling, with a rare compartment of *Sox2*-expressing tumour stem cells capable of driving long-term tumour growth and relapse *in vivo*. Whereas most stem cells in MB tumours are quiescent, a fraction of them express *Mki67*, a proliferation marker. The tumour sub-populations are highly genetically similar, suggesting that altered epigenetic states underlie the functional differences between them. We performed a comparative chromatin accessibility analysis of the quiescent and proliferating stem cell compartments of the tumour. Epigenetic profiling of these various sub-populations paired with expression data suggested that the transcription factor *Olig2* is among the regulators of the quiescent and activated stem cell states.
Olig2-expressing progenitor cells play a crucial role in mouse cerebellar development. By postnatal day 7, *Olig2* expression is downregulated in normal mouse development, but is found to persist in transgenic mouse models of SHH MB. Single cell transcriptomics reveal that *Olig2* is expressed in distinct subsets of cells expressing neural stem cell and cycling progenitor markers respectively. Further, transcription factor (TF) binding dataset analysis reveals that OLIG2 binding sites in mouse neural stem cells show a strong overlap with the binding sites of both stem cell TFs such as SOX2 and SOX9, as well as with differentiation-promoting TFs such as ASCL1, suggesting a multi-layered role of this TF in stem cells. We hypothesize that OLIG2 persists in cancer stem cells to promote abnormal proliferation, promoting tumour development.
Olig2 is frequently over-expressed in gliomas, however, its role in SHH MB tumour initiation is not well understood. We observed decreased proliferation and sphere-forming capacity in CRISPR-mediated *Olig2* knockout mouse MB tumour cells cultured in stem cell-enriching media, and also report a significant delay in their ability to form tumours compared to control in a subcutaneous allograft assay. Taken together, these data suggest that *Olig2* may play a role in promoting stem cell proliferation. Finally, we show that an *Olig2* inhibitor decreases the proliferation of mouse and human MB tumour cells. Our results may lead to a deepened understanding of tumour initiation in SHH MB and reveal a potential therapeutic target.

SYNDROMIC INTELLECTUAL DISABILITY CAUSED BY *DYRK1A* HAPLOINSUFFICIENCY DEMONSTRATES INVERSE DNA METHYLATION CHANGES FROM DOWN SYNDROME

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DYRK1A (Dual specificity tyrosine-phosphorylation-regulated kinase 1A) encodes a pleotropic protein kinase involved in critical

cell signalling pathways during brain development. It is located in the Down syndrome (DS) critical region and hence is a strong candidate gene for the cognitive deficits associated with DS. Pathogenic variants in *DYRK1A* are associated with a syndromic neurodevelopmental disorder. We have previously found that syndromic conditions caused by pathogenic variants in epigenetic regulatory genes show consistent patterns of DNA methylation in peripheral blood: *DNA methylation (DNAm) signatures*. DYRK1A is not an epigenetic regulator; however, due to its interactions with these proteins, we hypothesized that it might generate a unique DNAm signature. We compared DNAm in cases with pathogenic variants in *DYRK1A* (n=8) with age- and sex-matched neurotypical controls (n=24) using the Illumina Infinium MethylationEPIC array. Using linear regression, we identified 430 CpG sites differentially methylated in *DYRK1A* cases vs. controls (delta beta>10%, q<0.05). A machine-learning classification model based on the DNAm signature classified a validation cohort of *DYRK1A* cases (n=12) and controls (n=100) correctly, demonstrating 100% sensitivity and specificity. The model also classified four variants of unknown significance in *DYRK1A*, two classifying as pathogenic (with cases) and two as benign (with controls); all these classifications were congruent with the patient clinical phenotypes. We next investigated the *DYKR1A* DNAm signature sites in DS cases. We compared our *DYRK1A* cases with publically available 450k DS methylation data. At the *DYRK1A* signature sites, the DS cases clustered separately from the study's unaffected controls. Further, the methylation changes in the DS cases were in the opposite direction of the *DYRK1A* cases. In conclusion, these data represent the first DNA methylation signature of a non-epigenetic regulator, suggesting that DNAm signatures may result from functional impairment of many classes of genes which have not been previously considered. The opposing direction of DNAm changes in *DYRK1A* haploinsufficiency vs. trisomy suggests that the DNAm signature may reflect the functionality of DYRK1A. In future, these findings may be translated to diagnostic tests as well as novel therapeutic strategies.

DNA METHYLATION LEVELS REGULATE PD-L1 EXPRESSION IN MELANOMA SUBTYPES

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The programmed death-ligand 1 (PD-L1) receptor is an important immune checkpoint and is often upregulated in cancer cells to allow immune evasion. In melanoma, the patients with PD-L1 expression and absense of tumour infiltrating lymphocytes (TILs) (i.e. "constitutive PD-L1 or PD-$L1_{CON}$") show worse response rates and prognosis than patients with PD-L1 expression and the presence of TILs (i.e. "inducible PD-L1 or PD-$L1_{IND}$"). However, how PD-L1 expression is regulated in melanoma cells remains elusive. Understanding the mechanisms of how PD-L1 is regulated is important for predicting responses for anti-PD-L1 treatment and for developing new combinatorial therapies. We hypothesised that epigenetic state regulates constitutive and inducible PD-L1 expression in melanoma.

To address the hypothesis, we have generated whole-genome scale DNA methylomes (using reduced representation bisulfite sequencing) and transcriptomes (RNA-Seq) for patient derived melanoma cell lines (in PD-$L1_{IND}$ and PD-$L1_{CON}$ groups). We discovered extensive global hypomethylation in the constitutive lines, particularly pronounced in intergenic repeat regions and gene bodies. A high proportion of hypomethylated regions exhibited dichotomous methylation patterns indicating a common regulatory mechanism between the PD-$L1_{IND}$ and PD-$L1_{CON}$ lines. RNA-Sequening data indicated that the hypomethylated state of the PD-$L1_{CON}$ cells was correlated with higher upregulation of the differentially expressed genes at a global-scale and the upregulated genes were associated with cancer hallmark properties. The upregulated genes exhibited signatures of viral mimicry and cytosolic sensing of dsRNA similar to what has been observed after DNA methyltransferase inhibitor (DNMTi) treatment in cancers. DNMTi treatment increased PD-L1 transcription in the PD-$L1_{IND}$ cell lines. PD-$L1_{CON}$ samples showed greater resistance to DNMTi compared to PD-L1 negative samples. This was observed at protein-coding genes, transposable elements and long non-coding RNAs. Moreover, genes involved in the innate immune pathway was strongly increased upon DNMTi mediated hypomethylation in the PD-$L1_{IND}$ cell lines (FDR-adjusted P-value = 0.02), however there was no expression change in the PD-L1 positive cells (Adjusted P-value = 1.0). Furthermore, we found that the IRF1 transcription factor, which plays a critical role in the innate immune pathway and also binds to the PD-L1 promoter, is significantly upregulated upon DNMTi in the PD-L1 negative cell lines (Adjusted P-value = 0.01) but not in the PD-L1 positive cells (Adjusted P-value = 0.9). These results show that global hypomethylation levels regulate PD-L1 expression in melanoma. We believe these results are the first to show that DNA methylation levels play a role in regulating PD-L1 on melanoma and suggest they may have important implications for combined treatments targeting methylation (DNMTi) and PD1/PD-L1 (anti-PD1 antibodies).

COORDINATED ACTION OF HISTONE METHYLTRANSFERASES G9A/KMT1C AND NSD3/ WHSC1L1 REGULATE EXPRESSION OF *B*-GLOBIN GENES IN ERYTHROID CELLS

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Histone modifying enzyme G9a/KMT1C functions both as a co-activator and a co-repressor of transcription. In differentiated erythroid cells, G9a has been shown to mediate activation of adult *β globin* genes through its association with mediator complex, while it has been shown to mediate repression of embryonic gene E^{γ} through H3K27me2, H3K9me2 methylation and association with H3K4 histone demethylase JARID1a. In the present study we for the first-time report that G9a directly interacts with H3K36 methyltransferase NSD3 and this association is important for NSD3 mediated gene activation of adult *globin* genes in

differentiated erythroid cells. Doxycycline induced stable knockdown of NSD3, using two different shRNAs showed 60-70% downregulation of NSD3 both at transcript and protein levels and this decrease in NSD3 level was accompanied by significant downregulation of expression of *β^{maj}-globin* and *β^{minor}globin* genes, suggesting NSD3 is necessary for proper activation of adult *β-globin* genes. Furthermore, we observed a significant decrease in the global levels of di and tri H3K36 methylation upon NSD3 knockdown, which was further accompanied by decrease in the localization of both these marks on the *β^{maj}globin* gene thus, highlighting the necessity of these marks for proper activation of adult globin genes. While, there was no effect of NSD3 knockdown on localization of G9a and G9a mediated H3K27me2 and H3K9me2 methylation on *β^{maj} globin* gene, depletion of G9a leads to significant decrease in the localization of NSD3 and NSD3 mediated di and tri H3K36 methylation marks in the coding region of *β^{maj}globin gene, thereby* suggesting that G9a plays vital role in recruitment of NSD3 on the *β^{maj}-globin* locus. Overall our data suggests that coordinated action of histone methyltransferases G9a and NSD3 is necessary for proper activation of adult *β*-globin genes and NSD3 is a part of G9a-mediator co-activator complex.

EXPLORING EPISTATIC SUPPRESSION OF THE H3K27M ONCOHISTONE BY THE H3K36 METHYLTRANSFERASE ASH1L

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High-grade gliomas (HGG) is the most common group of malignant central nervous system neoplasms and is the leading cause of mortality and morbidity in children. Previously, we identified recurrent somatic mutation of histone H3 in pediatric HGG (pHGG) affecting two amino acids - lysine 27 to methionine (K27M) and glycine 34 to valine/arginine (G34V/R). H3K27M is a potent dominant negative mutant on the Polycomb Repressive Complex 2, resulting in global loss of the repressive H3K27 tri-methylation (H3K27me3) mark, and tumors in the midline of the brain. Conversely, H3G34V/R hinders H3K36 tri-methylation (H3K36me3), a mark associated with active transcription, resulting in tumours in the cerebral hemisphere. Such global perturbation of the H3K27me/H3K36me axis is implicated as the driving mechanism of gliomagenesis; however, whether this antagonism can be exploited to mitigate disease progression have not been investigated. Using a fly model, we determined that H3K27M results in loss of H3K27me3 concurrent with accumulation of H3K36me2 and eye malformation. Strikingly, knockdown of the H3K36me2 methyltransferase (KMT) *ash1*, completely rescues the *small eye* defect. The human ortholog ASH1L, is highly expressed in the brain, and ASH1L haploinsufficiency is associated with intellectual disability, myelination delay, and spine abnormalities. Examining targets of ASH1L in H3K27M glioma cells revealed very few binding sites, but striking specificity towards genes encoding brain organogenesis transcription factors. Using CRISPR-editing in H3K27M pHGG cells, we demonstrate that complete knockout of ASH1L causes mitotic defects. Transcriptome analysis revealed that loss of ASH1L catalytic activity results in down-regulation of cell cycling genes and concurrent up-regulation of telencephalon-specific genes, suggesting ASH1L may prevent forebrain specification in H3K27M glioma cells. Ongoing work will determine whether ASH1L can modulate the tumorigenic activity of H3K27M in patient-derived xenograft models. This study may yield insights into disease mechanism of H3K27M oncohistone with the potential of identifying H3K36 KMT as therapeutic targets for pediatric glioma.

INTERACTIONS OF DNA METHYLATION AND HISTONE LYSINE 36 AND 27 METHYLATION

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DNA methylation (DNAme) is an essential epigenetic mark for cell fate decisions and is strongly implicated in various diseases including cancers. Understanding DNAme distribution is critical to elucidate both normal development and pathogenesis. Histone marks have been found to be closely interacting with DNAme. Experiments showed that de novo DNA methyltransferase DNMT3B interacts with histone H3 lysine 36 tri-methylation (H3K36me3) via its PWWP domain and is recruited to deposit DNAme in gene body. However, how intergenic DNAme is established remained unclear. Recently, our group discovered an unexpected role of H3K36me2 in recruiting another de novo DNA methyltransferase DNMT3A to intergenic regions for depositing intergenic DNAme. Knocking out H3K36 methyltransferases NSD1 and NSD2 leads to H3K36me2 loss in intergenic regions, coinciding with the reduction of DNAme in the same regions. The DNAme landscape begins to take shape, providing mechanisms links among various diseases such as human developmental disorders (e.g. Sotos syndrome and Tatton–Brown–Rahman syndrome) and cancers (e.g. head and neck squamous cell carcinomas) which contain mutations in H3K36 methyltransferases (e.g. NSD1), DNMT3A, and/or K36M mutation. H3K27me3 is yet another histone mark closely related with DNAme. They tend to be mutually exclusive during mammalian development. But it remains elusive which is the cause. To investigate this further, we used three Glioblastoma (GBM) cell lines, namely BT245, DIPGXIII and HSJ019, which contain mutation K27M in histone H3.3. We have shown that the primary effect of K27M is to restrict H3K27me3 within unmethylated CpG islands (CGIs). We knocked out K27M in the three cell lines using CRISPR-Cas9 and profiled histone modifications and DNAme using ChIP-seq and whole-genome bisulfite sequencing (WGBS) respectively. We observed, consistently in three cell lines, that DNAme decreases in the regions where H3K27me3 increases upon the K27M-KO. However, the global DNAme levels can increase or decrease in the K27M-KO cell lines, suggesting different mechanisms for DNAme deposition in CGIs (or hypomethylated regions), genic and intergenic regions.

A DNA METHYLATION SIGNATURE FOR *EZH2* CAN IDENTIFY PATHOGENIC LOSS- VERSUS GAIN- OF- FUNCTION VARIANTS IN *EZH2* AND OTHER GENES OF THE PRC2 COMPLEX

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Weaver syndrome (WS), an overgrowth/intellectual disability syndrome (OGID), is caused by pathogenic variants in the histone methyltransferase *EZH2*. Using genome-wide DNA methylation data for 187 individuals with OGID and 969 controls, we show that pathogenic variants in *EZH2* demonstrate a highly specific and sensitive DNA methylation signature reflecting the phenotype of WS. This signature can be used to identify loss-of-function (LoF) and gain-of-function (GoF) missense variants in *EZH2,* as well as somatic mosaicism. The signature can also be used to classify sequence variants in *EED* and *SUZ12*, encoding other core components of the polycomb repressive complex-2 (PRC2). These data suggest that the *EZH2*-derived DNA methylation signature reflects the functionality of the PRC2 complex. Finally, we show that the signature is sufficiently robust to identify the presence of pathogenic variants in PRC2 complex genes in undiagnosed individuals with OGID. In summary, our findings demonstrate that *EZH2*-specific DNA methylation signature has clinical utility for diagnostic classification of sequence variants in *EZH2* and in identifying pathogenic variants in the more recently described OGID syndromes caused by loss-of-function variants in *EED* and *SUZ12*.

AGGRESSIVE DEDIFFERENTIATED ENDOMETRIAL CANCER CAN BE RECAPITULATED FROM CELL LINE MODELS WITH CHROMATIN REMODELING PROTEIN DEFICIENCIES AND TREATED WITH SYNTHETIC LETHALITY APPROACHES

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One of the most lethal yet rare subsets of uterine cancer is dedifferentiated endometrial carcinoma (DDEC). Less than 20% of patients diagnosed with DDEC survive compared to the over 80% of uterine cancer patients with high-grade endometrial cancer diagnoses. DDEC tumors possess both well-differentiated and undifferentiated regions. Previously, we demonstrated that 80% of the undifferentiated regions in DDEC lesions lack the expression of core chromatin remodeling proteins, SMARCA4 or ARID1A and ARID1B. We hypothesize that loss of these proteins, which are known regulators of transcription may lead to the induction and/or maintenance gene expression programs that drive dedifferentiation, metastasis and therapy resistance.
SMARCA4-deficient endometrial cancer (EC) cell line models were generated by CRISPR gene editing and were found to be less capable of self-renewal and anchorage-independent growth. *In vitro,* SMARCA4 knockout cells were found to be more senescent than their wild-type counterparts, possessing more positive beta-galactosidase stained cells and expressing higher levels of p21 and H3K9me3. Tumors formed from SMARCA4-deficient EC cell line models in immune-compromised mice recapitulated the mixed phenotype observed in patient DDEC lesions. Endometrial cancer cells lacking SMARCA4 expression were also found to be more sensitive to inhibition with clinically available therapeutics targeting CDK4 and EGFR. Synergistic effects upon combining therapies against CDK4 and EGFR will be evaluated. Exome sequencing, RNA-Seq, ChIP-Seq and ATAC-Seq will be carried out to elucidate whether it is the consequence of acquired mutations, changes in gene expression or alterations to nucleosome occupancy that contribute to cellular dedifferentiation in the context of DDEC.

DYSREGULATED IMMUNE GENE EXPRESSION IN MONOCYTES FROM AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) are a collection of heterogeneous developmental disorders that are characterized by deficiencies in social interactions and communication and increased repetitive behaviors and restricted interests. Immune dysfunction is a common co-morbidity seen in ASD, with innate immune dysfunction seen both in the brain and periphery. Previous work has identified significant differences in peripheral blood monocyte cytokine responses after ex vivo immune stimulation. However, an unbiased examination of gene regulation in response to different types of immune stimulants in ASD monocytes has not been examined. To examine how activation of different toll-like receptors (TLR) impacts gene expression in ASD, we cultured peripheral blood monocytes from 27 children with

ASD and 23 typically developing children and treated them with either lipoteichoic acid (LTA) or lipopolysaccharide (LPS) to activate LTR2 or 4 respectively. Following 24 hours of stimulation, we then performed RNA sequencing to profile mRNA responses between non-treated (NT), LTA and LPS treated samples for each diagnosis (control or ASD). We identified genes with differential expression in non-treated compared to stimulated cells for both control and ASD samples and then compared the responses between the two diagnoses. Both LPS and LTA induced expression of immune genes, with a subset that were differentially regulated in ASD compared to control samples. In response to LPS treatment, monocyte cultures from ASD patients showed a unique increase in genes within the Kegg pathway for Pathogenic *E. Coli* infection, a pathway that was not enriched in the controls. This pathway included elevated expression of key immune regulator genes such as FAS cell surface death receptor (FAS), nuclear factor kappaB (NFKB1), Interleukin Receptor Type1 (IL1R1), and TGF beta Kinase 3 (TAB3) in ASD. Notably, monocytes from control patients uniquely showed a consistent decrease in expression of genes associated with translation and rRNA metabolism in response to both LTA and LPS. A similar decrease was not observed in the ASD samples, suggesting a failure to properly regulate a prolonged immune response. Future work will validate the expression of the top target genes and pathways and identify key transcription factors (promoter motif analysis) that regulate the differential responses in monocyte activation. As monocytes are involved in early orchestration of the immune response, our findings will help elucidate the mechanisms regulating immune dysfunction in ASD and provide novel targets for future therapeutic development.

ACCURACY OF SEX PREDICATIONS IDENTIFICATION OF GENDERS ERRORS USING A TARGETED BISULFITE SEQUENCING APPROACH

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Several methods have been developed to predict sex using bisulfite arrays (450K and Epic) and whole genome sequencing. However, the efficiency of these methods, when applied to targeted bisulfite sequencing approaches is uncertain, we speculated that exiting methods may need to adapted or new developed for these new targeted sequencing approaches.
We recently completed the sequencing of 812 samples using Illumina's TruSeq Methyl Capture EPIC targeted bisulfite sequencing libraries. We evaluated the efficiency and performance of 5 different sex prediction methods including *minfi*[1], which compares the median methylation profiles of the X and Y chromosomes and clusters the sex chromosome methylation profile differentials (median(chr Y methylation) - median(chr X methylation)) into two groups (male and female) using K-means clustering. A second *minfi* approach uses the same K-means clustering but uses a ratio of the median coverage of X and Y. A third *minfi* uses K-means to cluster based on the ratio between the number of CpG sites on chromosome X vs. Y.
We then explored several novel approaches based only on CpGs that were well covered in the targeted data. First, we restricted attention to only CpG sites where >75% of individuals had read depths >5 on chromosomes X and Y. Then we calculated the proportion of these well-covered positions on the X chromosome where the methylation level was less than 0.01 and predicted female sex when this proportion was under 0.3 (New Method 1). Finally, we predicted female sex when less than 50% of the Y chromosome sites had a methylation proportion greater than 0.7 (New Method 2).
There were 10 samples that were identified by all predictors. Biological sex was determined for 94 samples, flagged by one or more method. Biological sex was confirmed using polymerase chain reaction (PCR) amplification of the X and Y, chromosomes. Performance was evaluated for males, females and the entire sample, with the best performance coming from *minfi* using the number of sites ratio test, with 100% prediction accuracy.

CONTINUOUS CHROMATIN STATE FEATURE ANNOTATION OF THE HUMAN EPIGENOME

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Sequencing-based genomics assays can measure many types of genomic biochemical activity, including transcription factor binding, chromatin accessibility, transcription, and histone modifications. Data from sequencing-based genomics assays is now available from hundreds of human cellular conditions, including varying tissues, individuals, disease states, and drug perturbations. Semi-automated genome annotation (SAGA) methods are widely used to understand genome activity and gene regulation. These algorithms take as input a collection of sequencing-based genomics data sets from a particular tissue. They output an annotation of the genome that assigns a label to each genomic position.
All existing SAGA methods output a discrete annotation that assigns a single label to each position. This discrete annotation strategy has several limitations. First, discrete annotations cannot represent the strength of genomic elements. Variation among genomic elements in intensity or frequency of activity of cells in the sample is captured in variation in the intensity of the associated marks. Such variation is lost if all such elements are assigned the same label. In practice, SAGA methods often output several labels corresponding to the same type of activity with different strengths, such as "Promoter" and "WeakPromoter". Second, a discrete annotation cannot represent combinatorial elements that simultaneously exhibit multiple types of activity. To model combinatorial activity, a discrete annotation must use a separate label to represent each pair (or triplet etc) of activity types. For example, intronic enhancers usually exhibit marks of both transcription and regulation. However, representing all possible combinations of activity types with discrete labels would

require a number of labels that grows exponentially in the number of activity types.
We propose a method that uses a Kalman filter state space model to efficiently annotate the genome with chromatin state features. That is, our method outputs a vector of real-valued chromatin state features for each genomic position, where each chromatin state feature putatively represents a different type of activity. Continuous chromatin state features have a number of benefits over discrete labels. First, chromatin state features preserve the underlying continuous nature of the input signal tracks, so they preserve more of the information present in the raw data. Second, in contrast to discrete labels, continuous features can easily capture the strength of a given element. Third, chromatin state features can easily handle positions with combinatorial activity by assigning a high weight to multiple features. Fourth, chromatin state features lend themselves to expressive visualizations because they project complex data sets onto a small number of dimensions.

ONCO-HISTONE H3.3G34W REDISTRIBUTES H3K27ME3 AND ALTERS THE CHROMATIN LANDSCAPE OF NEOPLASTIC CELLS IN GIANT CELL TUMORS OF THE BONE

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Recurrent heterozygous somatic mutations in histone H3 are found in a wide range of tumors including brain and bone tumors. Mutations affecting glycine 34 of H3.3 characterize a subset of cortical high-grade gliomas as well as the majority of giant cell tumors of the bone (GCTB). The substitution shows a remarkable degree of specificity to the anatomical compartment, for reasons that continue to elude researchers. In particular, glycine 34 to tryptophan mutations in H3.3 (H3.3G34W) characterize ~90% of giant cell tumors of the bone but have not been reported in brain malignancies.
However, it remains unclear how mutant H3 impacts the epigenome of neoplastic cells in GCTB and contributes to tumor formation. To address these questions, our group has developed an immortalized patient-derived cell line model of GCTB as well as isogenic edited counterparts via CRISPR/Cas9-mediated removal of the mutant allele.
Here, we present the combined analysis of the epigenome of these cellular models, via ChIP-seq profiling of H3.3 (H3.3WT and H3.3G34W), 4 histone marks (H3K36me2, H3K36me3, H3K27me3, H3K9me3), and transcriptomic profiling. We investigate whether mutant H3 induces a global remodeling of the epigenome. Principal component analysis reveals distinct transcriptomes and genome-wide deposition patterns of all profiled epigenetic marks between mutant and edited lines. Notably, we observe redistribution of H3K27me3 from intergenic to genic regions, as well as concomitant changes in H3K36me2 and H3K9me3 in response to altered H3K27me3. These epigenetic changes are accompanied by a change in transcriptional programs, with differential expression analysis revealing genes implicated in muscle contraction, actin binding, and extracellular matrix organization pathways, which may alter recruitment of the osteoclast/giant-cell component of the tumors that underlie the destructive lesions observed in GCTB.
Taken together, these results indicate that onco-histone H3.3G34W induces a global phenotypic change in GCTB stromal cells through the remodeling of the epigenome and a change in transcriptional programs that suggest a change in cell identity. This elucidation of how H3.3G34W alters the epigenome and the identity of the neoplastic cells is a first step toward the identification of a mechanism underlying H3G34 mutant-driven tumorigenesis.

IDENTIFICATION OF NOVEL PRIMORDIAL DWARFISM (PD)-LINKED GERMLINE MUTATIONS IN GINS3

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Primordial dwarfism (PD) is a developmental disorder characterized by pre- and postnatal growth delays and short stature, which results from germline mutations that disrupt formation of the pre-replication complex (Pre-RC) at replication origins. Pre-RC activation and initiation of DNA replication involves the assembly of CDC45 and the GINS subcomplex (composed of GINS1-4) forming the active CMG (CDC45-MCM-GINS) replicative helicase.
Recently, we identified novel gene mutations in a PD-affected patient (Patient 1), who is compound heterozygous for 2 different mutations in *GINS3*, causing D24G and R82Q substitutions. A second family was identified in which three PD-affected family members (Patients 2-4) are homozygous for a missense mutation in one of the same residues found in Patient 1 (D24N).
Patient 1 cells showed normal karyotyping and no signs of gross chromosomal instability. Cell cycle analysis however showed accumulation in S phase, which is also observed in cells harboring other PD-associated mutations in components of the Pre-RC. Lentiviral-mediated expression of wtGINS3 in primary patient fibroblasts rescued the cell cycle delay and slowed cell proliferation.
S phase stalling is indicative of DNA replication stress which triggers cell cycle checkpoints that prevent transition into G2/M phase only after DNA replication is complete. Increased phosphorylation of DNA-damage dependent cell cycle checkpoint proteins following hydroxyurea treatment in Patient 1 cells suggests enhanced sensitivity to DNA damage.

To assess the molecular consequence of the mutations, we evaluated the GINS3 interactome using proximity-based biotin identification of interacting proteins (BioID). Mass spectrometry analysis of biotinylated GINS3-interacting proteins found a reduction in the abundance of GINS3-GINS1 interactions in D24G-expressing cells, in comparison to the wt protein. There was also a reduction in the abundance of GINS3 interactions with other replication proteins. Immunoassays (immunoprecipitation or proximity ligation assay) confirmed the reduction in the interactions, likely caused by a reduced half-life in the mutant protein.
Modeling in yeast confirmed the altered cell cycle dynamics, reduced protein half-life and sensitivity towards replicative stress. These studies suggest that the GINS3 mutations identified in the present investigation are linked to growth abnormalities in Patient 1 and hence represents a novel PD-associated germline mutation.

SELLING DIRECT-TO-CONSUMER EPIGENETIC TESTS: A REVIEW OF CURRENT PRACTICES AND ETHICAL CONCERNS

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Building on recent findings in epigenomics, and following the path of direct-to-consumer (DTC) genetic testing giants like 23andMe and Ancestry.com, private companies have recently started to offer DTC testing for epigenetic variants associated with health and well-being, such as biological aging, smoke exposure and skin type, without supervision by a healthcare professional. While the ethical and legal implications of DTC genetic testing have received considerable attention over the past decades, other direct-to-consumer 'omic' tests have largely escaped scrutiny. We conducted a content analysis of the websites of five (5) DTC epigenetic testing companies (Chronomics, EpigenCare, Muhdo, MyDNAge, and TruMe), with a focus on their promotional messages, terms of services/and conditions, privacy policies and data governance policies. In this presentation, we will discuss the most salient ethical and legal issues arising with this new generation of DTC tests. We will touch to concerns regarding the medical relevance of the information provided, novel privacy risks, data protection challenges, and current absence of legal safeguards to prevent misuse of individual epigenetic information.

GENOME-WIDE DNA METHYLATION AND GENE EXPRESSION ANALYSIS OF NON-INVASIVE AND INVASIVE MELANOMA CELL LINES

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Melanoma is a serious form of skin cancer: New Zealand has among the highest rates in the world (50 per 100,000). Although survival has improved due to advances in therapies, metastasis is still a major cause of mortality in melanoma patients. Metastasis is very likely to be related to tumour microenvironment, e.g. hypoxia, but a better understanding of the underlying mechanisms is required. Previously, we, and others, demonstrated two main subgroups of gene expression, which correlate with non-invasive and invasive behavioral characteristics of melanoma cell lines. In the present study we hypothesized that differential DNA methylation could be associated with invasive and non-invasive melanomas. We carried out genome-wide DNA methylation analysis using reduced representation bisulphite sequencing (RRBS). A total of 39 differentially methylated fragments (DMFs) were identified in invasive versus non-invasive cell lines, with 20 DMFs being hypermethylated and 19 DMFs hypomethylated. Integrated analysis of DNA methylation and RNA sequencing (RNA-Seq) was carried out on these cell lines to identify possible relationships between DNA methylation and gene expression, which resulted in a total of 17 common differentially expressed genes associated with differential DNA methylation. Further analyses of these data are on-going. In summary, integrated analysis of DNA methylation and gene expression between invasive and non-invasive melanoma cell lines has revealed a list of differentially methylated and differentially expressed genes that are potentially involved in mechanisms associated with the invasive phenotype of melanoma.

UNCOVERING THE REGULATORY DOMAINS OF THE IMPRINTED-LIKE XLR GENE FAMILY USING CRISPR EPIGENOME EDITING

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The *Xlr* (X-linked lymphocyte regulated) gene family found on the X chromosome in mice has sparked a lot of interest in the last several years since the discovery that their regulation displays tissue-specific imprinted-like characteristics during early organogenesis and in later development. However, it is still unknown how or when they acquire these imprinted-like methylation signatures and their precise functions and regulatory domains are still to be determined. To study this phenomenon, we developed a mouse embryonic stem cell model (mESC *Dnmt1*$^{tet/tet}$) in which we can transiently deactivate *Dnmt1*, the enzyme responsible for DNA methylation maintenance, causing all methylation imprints to be lost since embryonic cells cannot recover these marks. We observed that all nine *Xlr* (*Xlr 3/4/5 a/b/c*) suspected promoter regions, that were initially all highly methylated, completely and permanently lose their methylation after *Dnmt1* deactivation causing a prolonged increase in expression, similarly to many methylated imprinted genes (e.g. *H19*/*Igf2*, *Nnat*, *Meg3*). Furthermore, the *Xlr* promoters were at first mostly depleted in histone modifications and then acquired active marks (H3K4me3, H3K27ac) following the loss of DNA methylation/gain in expression indicating that an epigenetic regulation mechanism implicating DNA methylation must be occurring. To investigate the *Xlr* family expression regulatory domains susceptible to DNA methylation, we transduce a CRISPR

epigenome editing system (dCas9-*Dnmt3a* or *TET1*) coupled to *Dnmt3a*, the enzyme responsible for *de novo* DNA methylation, or TET1, an enzyme that removes DNA methylation, to specifically methylate/demethylate different CpG dense regions of each *Xlr* individually and observe the effect on gene expression (RT-qPCR) and on histone modifications (ChiP-qPCR). This project will lead to the discovery of the precise expression regulatory domains of the *Xlr* genes needed to elucidate how they acquire a tissue-specific imprinting mechanism during development.

NORMAL BREAST TISSUE DNA METHYLATION AND RISK OF CONTRALATERAL BREAST CANCER: AN EPIGENOME-WIDE ASSOCIATION STUDY

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Introduction: Breast cancer risk is two to six times higher among breast cancer survivors compared to breast cancer risk in the general population. Given that alterations of DNA methylation patterns are the most common epigenetic aberrations in cancer and occur in cells during early breast cancer development and progression, the objective of the present study is to identify methylation changes in normal breast tissue that are associated with the incidence of a contralateral breast cancer (CBC) among breast cancer survivors. **Methods:** We conducted a nested case-control study based on a cohort of 757 patients diagnosed between 2000 and 2007 with a primary invasive hormone-receptor positive and non-metastatic breast cancer. Using an incidence density sampling scheme, 20 patients diagnosed with CBC (*in-situ* or invasive) at least 12 months after their first breast cancer (cases) were matched (1:1) with 20 patients who did not develop a CBC (controls) on year of surgery, age, menopausal status, family history of breast cancer, histologic type and HER2 status of the primary tumor and hormone therapy. Epigenome-wide DNA methylation in normal breast epithelium at least 1cm from the primary tumor was measured using the *Infinium450K* assay. Differentially methylated CpGs positions were identified using a robust linear regression and a strict Bonferroni correction for multiple testing ($P<2.4x10^{-7}$). Functional annotations (Gene Ontology, KEGG) were examined to determine biological plausibility and to identify pathways enriched for breast cancer risk-related differentially methylated genes. **Results:** As expected, no significant global methylation differences were observed between cases and controls. Of the 5591 identified CpGs (FDR Q<0.05), 45 passed strict Bonferroni correction, of which 31 were mapped to a known gene involved in cancer, immune or neurological diseases. Pathway analysis of these 31 genes identified four significantly (*P<0.01*) enriched pathways involving regulation of phosphate/phosphorus metabolic process. Two genes, *LHX2* and *SOD3*, harbored significant methylation changes at two different CpGs positions, while 10 genes, *PXDN*, *PODNL1*, *SVIL*, *PPP1R10*, *SOD3*, *CPA5*, *CLIC6*, *AP3D1*, *CAMK2N1* and *FARP1*, harbored significant methylation changes consistent with their differential expression in breast cancer (CPGA SAGE database). **Conclusion:** Once validated, these breast cancer risk-related differentially methylated genes could be used to enhance risk stratification for prevention of CBC in breast cancer survivors, and for developing new strategies for primary breast cancer prevention and treatment.

EPIGENOME DYSREGULATION RESULTING FROM NSD1 MUTATION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinomas (HNSCCs) are very common and deadly cancers that can develop in oropharynx, hypopharynx, larynx, nasopharynx, and oral cavity. Recently, mutations in epigenetics modifier genes, particularly the methyltransferase Nuclear Receptor Binding SET Domain Protein 1 (NSD1), have been implicated in HNSCC pathogenesis. Subsequently, our group has identified H3K36M mutations (*Lysine to methionine* mutations in histone H3 at the site 36) and demonstrated that NSD1 and H3K36M mutant HNSCCs form a distinct subgroup, characterized by specific DNA methylation patterns. We, and others, have argued that the main underlying mechanism of epigenetic dysregulation in the H3K36me tumors is a reduction in H3K36me2 levels, followed by a global reduction in DNA methylation. These observations, so far, have been mostly based on primary tumor data, bulk quantification of epigenetic modifications, or data resulted from genetic manipulation in mouse cell lines. Here, using CRISPR/Cas9 targeted genome editing technique, I knocked out NSD1 in three NSD1-wildtype HNSCC cell lines (Detroit562, Cal27, and Fadu) and generated isogenic cells. In addition, I further knocked out NSD2, a paralogue of NSD1, in one of the HNSCC NSD1-KO isogenic cell lines. Subsequently, genome-wide Chromatin Immunoprecipitation Sequencing (ChIP-Seq) and Whole Genome Bisulfite Sequencing (WGBS) techniques were utilized in order to finely characterize the differences in epigenetics characteristics of NSD1 (+) and NSD1 (-) HNSCC cell lines. So far, we demonstrated that a drastic and global decrease in DNA methylation at intergenic regions is directly associated with the lack of H3K36me2 mark in the same regions, followed by a concerted increase in the H3K27me3 mark (H3K27me3 changes reciprocally with H3K36me2). We are currently trying to reverse the mutation in one of HNSCC cell lines (SCC-4), which has a 1 base pair deletion in NSD1 gene, in order to determine whether the previously observed epigenetic modifications will be reversed or not. Recent studies show that presence or absence of mutation in NSD1 will significantly affect the outcome of different therapeutic approaches in HPV(-) HNSCC. Thus, understanding the specific function of NSD1 in HNSCC is of great importance for improving the treatment outcomes in HNSCC patients. Eventually, this project will more specifically investigate the role

of NSD1 and H3K36me2 mark and will uncover their correlation with H3K27me3 in HNSCC.

DATA COLLECTIONS ON THE ENCODE PORTAL

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The Encyclopedia of DNA Elements (ENCODE) is an ongoing international collaborative research project aimed at identifying all the functional elements in the human and mouse genomes. Data generated by the ENCODE consortium is freely accessible at the ENCODE portal (https://www.encodeproject.org/) that is developed and maintained by the ENCODE Data Coordination Center (DCC). In addition to the ENCODE consortium generated data, the portal hosts data from modENCODE, modERN, Roadmap Epigenomics Consortium and GGR projects.
There is no one correct way of mining the vast amount of data on the ENCODE portal, the faceted browsing interface, datasets cart feature, structured rich metadata and RESTful API, allow users to do it in ways that fit their specific needs. The ENCODE data corpus includes, but is not limited to the following data collections (1) mouse embryonic developmental series, unified set of chromatin state annotations tracking activity across developmental time and space, (2) functional map of protein-RNA interactions of RNA binding proteins (RBPs) encoded in the human genome, and the RNA elements they bind to across the transcriptome (ENCORE), (3) deep profiling of various human tissues from multiple donors, result of a GTEx and ENCODE collaborative project (ENTEx), (4) mouse and human reference epigenomes datasets compiled following guidelines set forth by the International Human Epigenome Consortium (IHEC).

CONSTRUCTING PHYLOGENIES IN EPIGENETIC CONTEXT

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Constructing accurate phylogenies from data is fundamental to understanding the evolutionary history and future trajectory of individuals in an ensemble. With the availability of reference epigenome maps, it has become possible to explore epigenetic relationships and drivers of epigenetic differences using phylogenetic analysis; the latter of increasing importance in clinical setting. However, the phylogenetic reconstructions are only meaningful if the choice of underlying evolutionary model is appropriate and have sensitivity to deviations from subtle assumptions about the model. Here we review common models used in phylogenetics for the working biologist, pointing out details like hetereotachy and clock assumption as well as the complications they introduce. We also survey related ideas like Phylo-HMM and Phylo-HMRF, which have been applied in analysis of chromatin architecture, and introduce phylogenetic algebraic geometry and invariants, as well as how they can be used to select between phylogenetic trees, illustrating their application in context of epigenetic data. Lastly, we explore how current models fail to capture all details in the phylogenetic analysis of epigenetic states and suggest an approach towards addressing this.

DELINEATING THE TRANSCRIPTIONAL REGULATORY ROLES OF RETROTRANSPOSONS IN HUMAN PLACENTA

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Approximately 40% of the human genome consists of retrotransposons, which are transposable elements that replicate via an RNA-mediated "copy-and-paste" mechanism. They are further divided into two classes: long terminal repeats (LTR) retrotransposons, also termed endogenous retroviruses (ERVs), and non-LTR retrotransposons, known as long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). If left unchecked, these sequences can potentially dysregulate gene expression, affect genome stability and induce mutations. Given their potential deleterious effects, retrotransposons are generally repressed by epigenetic mechanisms. Intriguingly, a subset of elements has been co-opted to become integral parts of our genomes, including functioning as cis-regulatory elements or as coding sequences. Interestingly, the placenta is particularly permissive to retrotransposon activities. Perhaps due to this feature, the best-studied example of ERV domestication is found in placental cells. The ERV-derived SYNCYTIN proteins play critical roles in the differentiation of syncytiotrophoblasts. Previous studies have also found retrotransposons to serve as placenta-specific enhancers or alternative promoters in both mouse and human genomes. These studies hint at the potential regulatory functions of retrotransposons in shaping placental transcriptomes and their putative involvement in placental disorders. Here we use state-of-the-art techniques to generate epigenomic and transcriptomic profiles from human placenta samples. We then perform integrative analysis to delineate the regulatory roles of retrotransposons in human placenta. We identify a specific AluJb element as a placenta-specific promoter for the LIN28B gene. Furthermore, we detect differentially expressed retrotransposons in placenta from preeclampsia patients. Our study provides insights into the role of retrotransposon in placental development and placental diseases.

GENOMIC AND EPIGENOMIC PROFILING OF PERITONEAL METASTASES TO IDENTIFY DRIVERS OF PROGRESSION FROM GASTROESOPHAGEAL ADENOCARCINOMAS

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Gastroesophageal adenocarcinoma (GEA) is a leading cause of cancer-related death worldwide with a five-year survival rate of less than twenty percent. The development of peritoneal metastasis (PM) is the most common progression of GEA and leads to an exceptionally poor prognosis with a median survival rate of less than four months. This poor prognosis results from the late presentation of the disease, the inability to surgically resect the cancerous lesions and the absence of therapeutic options. Emerging data suggests that extensive epigenetic reprogramming accompanies the progression of cancers to metastasis. We are currently profiling 18 matched PM patient trios (normal, primary and peritoneal metastasis) for H3K27Ac and H3K27me3, epigenetic markers of active and inactive chromatin, by ChIPmentation to identify gained and lost regulatory elements and their corresponding genes. We have currently profiled 5 patient samples (1 PM, 2 primary GEA, and 2 normal gastric mucosa) and have identified several PM-specific activated and inactivate genes. For example, we identified *HOXB13* as activated and *CDHR3* as inactivated only in our PM sample. Furthermore, by analyzing the gained regulator elements, we can identify enriched transcription factor (TF) binding motifs, suggesting expression of the corresponding TF. Using published H3K27Ac ChIP-seq data, we identified several TFs potentially involved in metastasis, including SALL4. Activation of *SALL4* was also observed in our profiled PM sample suggesting a role in the progression to GEA. We are also profiling our PM patient trios using whole exome sequencing (WES). To date, we have completed sequencing of 5 patient matched trios (n=15). Interestingly, we have discovered an enrichment of somatic mutations within several known cancer genes previously reported to be involved in cancer progression and metastasis, such as *CDH1* and *MYH9*. In addition, we have identified several novel genes that are enriched with somatic mutations and potential drivers in the progression of GEA to PM. Currently, molecular information is not incorporated into the management of GEA and patients with PM are considered palliative. The identified molecular drivers of PM will represent not only new therapeutic targets for the treatment of this disease but will also reveal the patient population most likely to benefit from alternative treatment strategies.

OBSESSIVE-COMPULSIVE DISORDER AND ATTENTION-DEFICIT/HYPERACTIVITY DISORDER: DISTINCT ASSOCIATIONS WITH DNA METHYLATION AND GENETIC VARIATION

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Background: A growing body of research has demonstrated associations between specific neurodevelopmental disorders and variation in DNA methylation (DNAm), implicating this molecular mark as a possible contributor to the molecular etiology of these disorders and/or as a novel disease biomarker. Furthermore, genetic risk variants of neurodevelopmental disorders have been found to be enriched at loci associated with DNAm patterns, referred to as methylation quantitative trait loci (mQTL).
Methods: We conducted two epigenome-wide association studies in individuals with obsessive-compulsive disorder (OCD) or attention-deficit/hyperactivity disorder (ADHD) (aged 4-18 years) using DNA extracted from saliva. DNAm data generated on the Illumina Human Methylation 450K array was used to examine the interaction between genetic variation and DNAm patterns associated with these disorders.
Results: Using linear regression followed by principal component analysis, individuals with the most endorsed symptoms of OCD or ADHD were found to have significantly more distinct DNAm patterns from controls as compared to all cases. This suggested that the phenotypic heterogeneity of these disorders is reflected in altered DNAm at specific sites. Further investigations of the DNAm sites associated with each disorder revealed that despite little overlap of these DNAm sites across disorders, both disorders were significantly enriched for mQTL within our sample.
Conclusions: Our DNAm data provide insights into the regulatory changes associated with genetic variation, highlighting their potential utility both in directing GWAS and in elucidating the pathophysiology of neurodevelopmental disorders.

ROLES OF THE IMPRINTED GENE *MEST* IN DEVELOPMENT

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

MEST shares homology with epoxide hydrolases. It has a conserved α/β-hydrolase fold, shared by a large family of hydrolases, and is localized in the endoplasmic reticulum (ER). *Mest* is highly expressed in embryonic and extraembryonic mesoderm during development, including the placenta. Recent studies have also shown that MEST may act as an inhibitor of the Wnt pathway.

To address the function of *Mest* in embryonic growth, we will utilize our *Mest* KO mutant mouse line previously generated. The KO line has been brought onto C57BL/6J reference strain background for further studies. We have observed that the growth retardation phenotype seen *in utero* from E11.5 to E17.5 is recapitulated on the C57BL/6J background. *Mest* KO embryos are approximately 20% smaller than their WT littermates. The role of MEST in regulating the Wnt pathway has been tested *in vitro* using the TOPFLASH reporter assay in WT and KO embryonic fibroblasts. There is no statistically significant difference in Wnt pathway induction between genotypes. There are also no structural or molecular abnormalities in the mutant placentae based on histology and RNA *in situ* hybridization for placental markers. However, KO E17.5 placentae exhibit lowered triglyceride levels, suggesting MEST may play a role in lipid biosynthesis.

To identify the substrate(s) of MEST, we have developed an unbiased substrate-trap approach taking advantage of the characteristic 2-step reaction of some α/β-hyrdolases. We have generated mutations in the catalytic triad that prevents hydrolysis of the covalent substrate-MEST reaction intermediate using an episomal system in HEK293T cells. The substrate can then be identified by mass spectrometry. Further experiments characterizing MEST topology within the ER using the Split-GFP system are currently being performed.

MEST may play a role in lipid metabolism in the placenta, and we hypothesize that loss of this function could be responsible for the growth retardation phenotype in KO mice. Characterization of MEST protein architecture, and putative substrates will provide insight into the function of *Mest* in development. This will provide important information on the evolutionary significance of the conserved imprinting of *Mest* in placental mammals and may also impact the management of Silver-Russell patients.

EPIGENETIC REGULATION OF ENHANCER REGIONS IN BREAST CANCER CELLS IN RESPONSE TO PTEROSTILBENE

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Breast cancer is the most common cancer and the second leading cause of cancer death among women. It has been suggested that at least 30% of breast cancer cases are caused by epigenetic alterations. Interestingly, certain dietary compounds such as polyphenol found abundantly in blueberries have been shown to regulate gene expression and reverse tumour progression by altering epigenetic patterns. Our group has proposed the involvement of DNA methyltransferase 3B (DNMT3B) and transcription factor OCT1 as vital players in polyphenol-mediated targeting of oncogenes. We have also identified enhancer regions as important regulatory sites with altered DNA methylation in response to polyphenols. However, the genome wide effects of pterostilbene-mediated alterations in the occupancy of DNMT3B and OCT1 in enhancer regions of oncogenes remains to be elucidated.

In this study, following ChIP-Seq analysis of highly invasive breast cancer cells (MCF10CA1a) treated with 7 μM pterostilbene for 9 days, we discovered that pterostilbene treatment leads to altered occupancy of DNMT3B and OCT1 at 111 and 323 enhancer regions, respectively. DNMT3B and OCT1 demonstrated overlap in 78 regions corresponding to 26 genes. In those 26 candidate genes, peaks of increased in binding of DNMT3B and decreased in occupancy of OCT1 were within 500 bps. In addition, H3K36me3 enrichment was measured to indicate decrease in transcriptional activity within the same regions. QPCR and pyrosequencing were performed to assess gene expression and methylation status of selected genes, respectively.

We identified 6 candidate genes whose enhancer regions demonstrated increased binding of DNMT3B, decreased occupancy of OCT1 and lower enrichment of H3K36me3 in MCF10CA1a upon pterostilbene treatment compared with control untreated cells ($p<0.05$), indicating gene silencing. Indeed, QPCR confirmed down-regulation of 4 of those candidate genes namely *DANT2, TNNT2, LINC00910* and *PITPNC1.* The identified candidate genes were grouped into two types, oncogenes and long non-coding RNA class genes with potentially oncogenic functions. Using pyrosequencing, we detected methylation changes in *DANT2*, *TNNT2* and *LINC00910*. The enhancer region of *DANT2* encompassing 9 CpG sites showed 5-10% increase in methylation throughout the region upon PTS treatment. Oncogene *TNNT2* was hypermethylated by 14% at CpG 1 and LINC00910 by 15% at CpG 2. These changes coincided with 41%, 87% and 92% down-regulation of *DANT2*, *TNNT2* and *LINC00910* expression upon pterostilbene treatment, respectively.

This work provides novel insight into the mechanisms of dietary polyphenols in driving epigenetic changes at enhancer regions in breast cancer cells. Such epigenetic activities are of importance in cancer prevention and therapy.

EPIGENETIC DYNAMICS IN RELAPSE OF B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Disease relapse of B cell acute lymphoblastic leukemia (B-ALL) is present in 40-75% of adult patients and remains a significant clinical challenge. Like many cancers, relapse of B-ALL can stem from the growth of a pre-existing subpopulation of cells present at diagnosis that remains throughout therapy due to its genetic and/or epigenetic profile. In this work, we combine targeted genome, transcriptome, targeted bisulfite, and chromatin accessibility sequencing data from five patients at diagnosis and relapse, as well as patient-derived xenografts, to study the genetic and epigenetic dynamics of relapse, and how they operate in concert with one another.
Using similarity network fusion, a method for dataset integration, we find that DNA methylation better discriminates relapse-fated subclones than RNA abundance and chromatin accessibility. While there are no recurrent differentially methylated regions (DMRs) between diagnosis and relapse across all patients, we find that each patient has hundreds to thousands of DMRs between the two disease stages. In two individuals, a majority of DMRs are consistent with the mutation profiles of the relapse-fated subclones, while in the remaining three the majority of DMRs are independent of these subclones. Notably, some of these methylation changes oppose the changes that occur over the course of normal B cell differentiation, producing a methylation profile more like progenitor cells.
These data suggest that relapse is not necessarily strictly driven by genetic factors and that B-ALL relapse may result from, in part, epigenetic changes that produce a more stem-like phenotype in a subpopulation of cells at diagnosis.

CHROMATIN EXHIBITS SOLID-LIKE BEHAVIOUR IN LIVING CELLS

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Recently, our understanding of chromatin folding beyond the 10 nm extended nucleosome structure has changed. One of the emerging themes in chromatin folding is that chromatin might be organized by a liquid unmixing and phase separation event. We critically tested this hypothesis. We find that condensed chromatin does not exist in the predicted liquid state within living cells. Rather, condensed chromatin behaves as a solid in living cells. We next tested the state of chromatin outside of condensed regions and contrary to our expectations, we found that this chromatin also behaves as a solid rather than the expected liquid state. Subsequently, we tried to generate liquid-like behaviour through overnight treatment with histone deacetylase inhibitors or laser microirradiation. Transmission electron microscopy revealed that cells treated with histone deacetylase inhibitors were comprised mostly of dispersed 10 nm fibers. Despite this decondensed state, both experiments continued to show that chromatin has solid-like behaviour. Consequently, we readdressed the in vitro analyses and observed that both chromatin fragments isolated from nuclei and reconstituted chromatin templates using only core histones, similar to what was recently published, also show reveal that the chromatin is in a solid state. Surprisingly, our results show that, while the histone amino termini contribute to the extent that the chromatin fibers are able to interact with themselves to form visible condensates, all of the cellular chromatin that we can detect behaves as a solid. Finally, we assessed the mobility of individual early S-phase replication foci, which are enriched in euchromatic regions of the genome, and found evidence for coordinated movement, reflecting a solid nature to the interphase chromosome and explaining the mobility reported when tracking single gene loci.

DNA METHYLATION-DRIVEN CHANGES IN BINDING OF TRANSCRIPTION FACTORS

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Methylation of cytosine, 5-methylcytosine (5mC), is the most common DNA modification and has an important role in the regulatory landscape of the cell. The presence of 5mC at the transcription factor (TF) binding sites can change the strength of the TF binding to DNA. Traditionally, 5mC was thought to only have a negative impact on TF binding. However, recent *in vitro* studies of TFs suggest that the TF binding preferences for methylated cytosine is heterogeneous, and, depending on the TF and the position of modification, 5mC can either increase or decrease binding of the TF. Interestingly, this heterogeneity is present even within TF families.
Research shows that the effect of 5mC on TF-DNA interaction is more complex than previously believed. The specific effect of these interactions is still unknown for most TFs *in vitro* and, more importantly, *in vivo*. In this project, we aim to model the interplay between TF, methylation and DNA. We present a model that combines base-resolution maps of DNA methylation with DNA sequence and accessibility to quantitatively predict the *in vivo* binding strength of TFs, as measured by ChIP-seq. As a proof of concept, we trained and evaluated this model on the binding preferences of CTCF in the HepG2, GM12878, HEK293, and K562 cell lines, using publicly available chromatin accessibility data (DNA hypersensitivity I assay) and base-resolution cytosine methylation map (Whole Genome Bisulfite Sequencing) of each cell line. We found that the predictions of our model highly correlate with experimental TF binding strength in every cell line. Importantly, we observed a negative effect on binding by the

presence of 5mC sites at specific positions of the CTCF binding sequence, which aligns to previous models of CTCF-DNA interaction. Interestingly, we also found that even when the chromatin accessibility of a particular genomic region does not change across cell lines, differences in CTCF binding can still be predicted based on the change in DNA methylation alone.
These results suggest that it is possible to learn the effect of DNA methylation on TF binding from *in vivo* binding data and highlight the applicability of our method for predicting cell type-specific TF binding by combining DNA sequence with epigenomic features. In our future work we will expand these analyses to other TFs in order to systematically study the relationship between DNA modifications and DNA binding across TF families.

CRISPRI SCREEN OF RISK-ASSOCIATED CIS-REGULATORY ELEMENTS REVEALS 3D GENOME DEPENDENT CAUSAL MECHANISMS IN PROSTATE CANCER

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BACKGROUND: Prostate cancer is one of the most heritable diseases to date. Hundreds of single nucleotide polymorphisms (SNPs) have been identified by genome-wide association studies (GWAS) to confer risk of prostate cancer in men. Most prostate cancer associated risk SNPs do not directly alter gene codons, rather modulate cis-regulatory elements (CREs) such as enhancers. The primary objective of this study was to perform a systematic essentiality screening of prostate cancer risk associated CREs.
METHODS: We previously pinpointed 270 CREs that harbour at least one risk SNP in prostate cancer. In this study, we targeted these CREs using dCas9-KRAB complex (CRISPRi) guided by 5,571 sgRNAs in three prostate cancer cell lines - LNCaP, V16A and 22Rv1.
RESULTS: The screen identified 98 CREs essential for growth of at least one cell line. Interestingly, essential CREs are significantly enriched in the gene desert region of 8q24.21. The most essential CRE is an enhancer harbouring the SNP rs11986220, which increases the risk for prostate cancer by up to 1.8-fold. Suppression of this enhancer significantly reduces cell proliferation and tumor growth in LNCaP and V16A models. RNA-seq analysis identifies MYC, an important oncogene, to be its primary target gene. However, this enhancer neither confers essentiality nor regulates MYC in 22Rv1 cells, despite having almost identical epigenetic profiles as in LNCaP cells. Further investigation reveals that a CTCF binding site unique to 22Rv1 intervenes the MYC promoter-enhancer interaction in this cell line. We performed 3C, HiC and H3K27ac HiChIP assays to establish that the enhancer interacts with MYC promoter only when this CTCF site is deleted in 22Rv1 cells. Intriguingly, this CTCF site is also found variable among primary prostate cancer patients, and especially, the SNP rs11986220 is an eQTL for MYC only in patients with low deposition of CTCF at this locus.
CONCLUSION: Our study reveals that CRISPRi is an efficient technique to perform systematic functional analysis of CREs. We thus discover that the interaction between MYC promoter and rs11986220-containing enhancer is governed by CTCF-mediated 3D genomic structure, and the causal effect of rs11986220 is variable among patients depending on CTCF binding in this locus. This unveils a novel regulatory mechanism in human genome and may present a paradigm shift for current target-gene analysis of GWAS loci by incorporating 3D genome variability.

ENZYMATIC METHYL-SEQ: NEXT GENERATION METHYLOMES

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DNA methylation is important for gene regulation. The ability to accurately identify 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) gives us greater insight into potential gene regulatory mechanisms. Bisulfite sequencing (BS) is traditionally used to detect methylated Cs, however, BS does have its drawbacks. DNA is commonly damaged and degraded by the chemical bisulfite reaction resulting in libraries that demonstrate high GC bias and are enriched for methylated regions. To overcome these limitations, we developed an enzymatic approach, NEBNext® Enzymatic Methyl-seq (EM-seq™), for methylation detection that minimizes DNA damage, resulting in longer fragments and minimal GC bias.
Illumina libraries were prepared using bisulfite and EM-seq methods. Libraries generated with NA12878 DNA inputs ranging from 10 ng to 200 ng were sequenced using Illumina's NovaSeq 6000. Reads were adapter trimmed (trimadap) and aligned to GRCh38 using BWAMeth. Aggregate metrics like GC bias and insert size distribution (Picard) were assessed before evaluating methylation status of individual Cs (MethylDackel). MethylKit was used for correlation analysis. EM-seq libraries have longer inserts, lower duplication rates, a higher percentage of mapped reads and less GC bias compared to bisulfite converted libraries. Global methylation levels are similar between EM-seq and whole genome bisulfite libraries (WGBS) indicating overall detection of methylated Cs is similar. However, CpG correlation plots demonstrated higher correlation coefficients indicating that EM-seq libraries are more consistent than WGBS across replicates and input amount. GC Bias and dinucleotide distribution showed that EM-seq has more even dinucleotide representation compared to the AT rich representation observed for WGBS. EM-seq's more even coverage allows for a higher percentage of CpGs to be assessed leading to more consistent evaluation of methylation across key genomic features (TSS, CpG island, etc.). EM-seq is more robust than WGBS, works over a wide range of DNA input amounts, has superior sequencing metrics, and detects more CpGs.

CROSSTALK BETWEEN HISTONE H3K27, H3K36 AND DNA MODIFICATIONS IN HIGH GRADE GLIOMAS

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Perturbations of chromatin organization have been implicated in multiple human disorders and cancer. For instance, counteracting Polycomb Repressor Complex 2 (PRC2) activity by H3K27M mutation is involved in pediatric brain tumors, specifically the High-Grade Gliomas (HGG) diffuse intrinsic pontine gliomas (DIPGs) and supratentorial glioblastoma multiforme (GBMs), in which H3K27me3 silencing mark is greatly reduced genome wide. Besides, direct mutations in *EZH2*, the catalytic subunits of PRC2, are found in Weaver Syndrome, a disorder characterized by an overgrowth phenotype in childhood. Similarly, mutations in the *NSD1* gene, expressing an enzyme that mediates mono- and di-methylation of histone H3 at lysine 36, are responsible for Sotos Syndrome, another overgrowth pathology in childhood. NSD1 alterations, along with the H3K36M mutation which is associated with reduced activity of H3K36 methyltransferases, have also been identified in head and neck squamous cell carcinomas (HNSCCs). Our group has shown convergent downstream effects of *NSD1* and H3K36M mutations, resulting in specific reduction of H3K36me2 and DNA methylation in intergenic regions, together with reduced targeting of DNMT3A (DNA methyltransferase) in those regions. Furthermore, Tatton-Brown-Rahman syndrome, another childhood overgrowth syndrome, is defined by germline mutations in *DNMT3A*. Although a relationship between H3K27 (PRC2) and H3K36 (NSD1/2), and now DNA methylation has been proposed in different studies, it is not clear how these PTM influence each other and how the tight regulation of these players influence chromatin homeostasis and can lead to different pathologies with phenotypic similarities. Since methylation of H3K36 residues restricts spreading of H3K27 silencing marks, our main 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. For that purpose, we use human pediatric HGG cell lines, harboring H3K27M and characterized by restriction of H3K27me3 mark to CGIs, which is thought to lead to cell differentiation stalling causing them to indefinitely multiply. We decided to analyse the epigenetic consequence of removing H3K36 mark in intergenic regions by performing CRISPR knock-out of *NSD1* and *NSD2* (NSD1/2 dko) in the BT245 cell line (H3K27M). We observe significant reduction of H3K36me2 mark and DNA methylation in intergenic regions in NSD1/2 dko cells. We hypothesise that loss of H3K36 methylation would provide new nucleosomal substrate for PRC2 and indeed, our data show increased H3K27me2 spreading in silencing regions in absence of NSD1 and NSD2. In addition, we observe that mutating *NSD1* and *NSD2* causes dramatic up-regulation of genes related to metabolism compared to unedited cells. We are now testing the effects of these changes, for example, by exploring if the NSD1/2 dko cells have restored ability to differentiate. We are also testing the tumorigenesis of the NSD1/2 dko cells in mice side by side with parental BT245 injection. Studying the relationship involving H3K27, H3K36 and DNA methylation is of great importance as it could potentially be used for specific or combinational therapy, for example by using or developing epigenetic inhibitors limiting or abrogating H3K36me2 specifically in intergenic regions that could partly restore spreading of H3K27 methyl mark and silencing of proper genomic regions in pediatric HGG.

IDENTIFICATION OF FACTORS INVOLVED IN REGULATING DNMT3B EXPRESSION IN PLURIPOTENT CELLS

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During early embryonic development, cells in the blastocyst's inner cell mass called the epiblast undergo mass *de novo* DNA methylation as the blastocyst undergoes implantation. The epiblast gives rise to the somatic cells that form the mature organism, and the genome-wide DNA methylation pattern acquired during peri-implantation development is largely maintained throughout life. DNMT3B is one of two enzymes that mediate *de novo* DNA methylation and is dramatically upregulated during the peri-implantation period. We are using human pluripotent stem cells (hPSCs) to determine how DNMT3B is upregulated. We have identified putative enhancer regions upstream of the DNMT3B TSS, which are enriched for H3K27ac, H3K4Me1, RNAPII, Med1 and p300, and shown that these regions elicit enhancer activity through luciferase reporter assays. CRISPR interference-mediated heterochromatinization of these candidate enhancer regions reduces DNMT3B expression, again consistent with their being enhancers that regulate DNMT3B. We also determined that ZIC2 – a transcription factor highly expressed in primed (post-implantation-like) hPSCs and co-expressed with DNMT3B during primate development, binds to the putative enhancers upstream of the DNMT3B locus in hESCs. Furthermore, through luciferase reporter assays, we have shown that ablation of ZIC motifs within these candidate enhancers results in reduced enhancer activity. Overall, through this and future studies, we hope to identify the cis-regulatory elements and trans-acting factors important in controlling DNMT3B expression in humans.

IDENTIFICATION OF FACTORS INVOLVED IN THE CHROMATIN BINDING OF POLYCOMB GROUP PROTEINS ACROSS THE CELL CYCLE

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A critical part of cell identity is the establishment and maintenance of gene expression patterns. Polycomb group (PcG) proteins act on chromatin to maintain gene repression through cell cycles (epigenetically). During mitosis, chromatin structure is greatly altered by transcription repression, chromatin condensation and the release of many chromatin proteins. A question then arises:

how is PcG repressive function maintained through mitosis? In interphase, PcG proteins are bound to their chromatin targets. During mitosis, most PcG proteins are released from chromatin, but a fraction remains bound. According to the mitotic bookmarking hypothesis, this fraction acts as a set of markers to guide PcG protein recruitment at the end of mitosis to maintain the gene expression profile. However, we do not know how this recruitment takes place, or how a fraction of PcG proteins is retained on chromatin.
Using an imaging based high throughput RNAi screen, we identified genes that increase or decrease PcG protein binding to chromatin in mitosis. Specifically: genes were knocked down in *Drosophila* S2 cells using dsRNA. Cells were treated with colchicine to increase the mitotic index and processed for immunofluorescence against the PcG protein Polyhomeotic (Ph). Screen hits (increased or decreased Ph binding to chromosomes) were identified using median absolute deviation and quartile-based analyses. We identified 27 hits from 568 genes tested in duplicate. Multiple genes encoding proteins in the same complexes are hits, providing confidence that hits are *bona fide*. Flow cytometry analysis of extracted dsRNA treated cells is being used to confirm that the hits affect Ph levels on chromatin.
We now aim to determine how the identified genes control PcG protein binding in mitosis. Western Blot analysis of dsRNA treated cells indicates that most hits do not influence the level of Ph protein, but rather its distribution within the cell. ChIP-qPCR confirms that Ph chromatin binding is decreased in cells treated with dsRNA against Ph. ChIP is now being used to test whether screen hits influence the level of Ph binding to chromatin in mitosis and/or the sites it binds to. Other functional analyses of confirmed hits will include testing for protein-protein interactions between hits and PcG proteins, and for effects on expression of PcG target genes.
How chromatin-based information is propagated through mitosis is not known but is fundamental to epigenetic inheritance. The identified genes may uncover new links between PcG proteins and the cell cycle, which may reveal how and why most PcG proteins are released from chromosomes in mitosis, and other mechanisms involved in transmission of epigenetic information through mitosis. PcG proteins and their epigenetic functions are widely conserved and are implicated in both development and disease, including many cancers. Understanding basic mechanisms of epigenetic inheritance thus has long term implications for human health.

THE EPIGENOME REFERENCE REGISTRY (EPIRR)

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The IHEC consortium aims to produce more than 1000 reference epigenomes and integrate them in large analyses such as the EpiMap project. However, the data supporting these epigenomes are stored in a variety of different public archives, depending on the nature of the data and security requirements of the samples. The EpiRR reference registry (http://www.ebi.ac.uk/vg/epirr), acts as a central reporting and accessioning point for this distributed data, providing IHEC partners and third party scientists a single point of entry to locate all IHEC datasets across public archives. We have extended EpiRR to store metadata, so as to provide a central location for IHEC metadata too, opening up the possibility of searching for IHEC datasets in a comprehensive and consistent fashion. In effect, EpiRR can now read the metadata from the public archive submission and distribute it, thus simplifying the process of IHEC data submissions. Already, we are supporting the EpiMap project by providing regular statistics on the metadata update efforts across all of IHEC.

THE PLACENTA IS NOT AN ASEXUAL ORGAN: SEX-SPECIFIC PLACENTAL DNA METHYLATION SIGNATURES

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Background: Fetal sex is associated with differences in development and risk of certain perinatal complications throughout gestation. For example, while males are often larger than females, they are also more likely to be born premature. These differences can be partially attributed to sex-specific function of the placenta, an organ critical for development: during pregnancy it transports nutrients required for fetal growth, removes waste, and regulates the fetal environment. Molecular features such as DNA methylation (DNAme) are associated with gene expression patterns and may therefore be related to sex-specific placental function and fetal development. As factors like XCI and fetal sex-hormone disparities have the potential to influence sex-specific DNAme and gene expression patterns at autosomal loci also, it is important to evaluate sex differences genome-wide. **We hypothesize that, in addition to differences on the X-chromosome, sex specific autosomal DNAme exists in the placenta and is related to sex differences in placenta function and fetal health.**
Methods: We compiled Illumina 450K DNAme array data for 341 normal term placentae from 8 public datasets (51% female). Autosomal DNAme data were filtered and normalized, and we identified sex-specific autosomal DNAme by linear modelling, thresholding for CpG sites at least 10% differentially methylated (DM) by sex, and satisfying a false discovery rate (FDR) <0.01. DM at autosomal loci was confirmed in two additional datasets (n=116, 47% female).
Results We identified sex-specific DNAme at 166 of 324,104 autosomal sites tested (>10% DM, FDR<0.01), DM at 90% of these loci replicated in 116 samples from 2 independent datasets. Many DM autosomal sites were in genes previously associated with pregnancy complications, placentation, or immune function, such as 7 male-hypermethylated sites in the promoter of *ZNF300* (preeclampsia, fetal growth restriction, and has binding sites in *IL-2* and *IL2RB*), male hypermethylation at 3 sites in the *SPON1* gene body (preeclampsia), and female hypermethylation at 2 gene body sites in *ARHGAP15* (found in placental exosomes). Further, the 84 genes associated with the 166 DM sites showed enrichment in chemokine-mediated signaling pathways, eosinophil chemotaxis,

peptide cross-linking, and cornification, suggesting a potential role for immune pathways in placental sex differences. Intriguingly, hierarchical clustering of all samples based on DNAme at these 166 loci arranged male and female samples not into discrete groups, but across a continuum, suggesting sex-specificity, rather than sexual dimorphism, in autosomal DNAme profiles.
Conclusions: The detected sex-specific autosomal DNAme signatures are subtle but robust, and are consistent with sex differences in perinatal development. These analyses provide a means of identifying candidate genes/regions involved in differential placental function and fetal development.

DYNAMIC EPIGENETIC REGULATION DURING B-CELL MATURATION AND TRANSFORMATION TO CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Chronic Lymphocytic Leukemia (CLL) is a malignancy characterized by the progressive accumulation of long-lived CD5$^+$ B-lymphocytes in peripheral blood, bone marrow and secondary lymphoid organs. During transformation, CLL cells undergo extensive alterations in transcription and DNA methylation, which recapitulate the heterogeneity and clonal evolution of this disease. However, it is not yet understood which epigenetic mechanisms specify the gene regulatory states during B-cell maturation and how these are altered during the transformation to CLL. To study these mechanisms, we analyzed chromatin state-, DNA methylation-, DNA accessibility- and gene expression datasets from primary CLL cells and normal B-cell controls. We hypothesize that pathogenic epigenetic states contribute to the regulatory dysfunction that drives CLL transformation and could serve as novel prognostic markers and therapeutic targets.
Methods: To reveal potential drivers of CLL, we generated human reference epigenomes for primary CD19$^+$ cells from 16 CLL patients and for germinal centre B-cells from 6 healthy donors. In addition, we analyzed reference epigenomic datasets from six B-cell subtypes in 75 healthy individuals and CD19$^+$ cells from 107 CLL patients generated as part of the BLUEPRINT program as well as bisulfite- and RNA-sequencing data from a cohort of 116 CLL patients from Large-Scale CLL Genome Analysis (phs000435.v3.p1) obtained from dbGAP.
Results: Consistent with previous reports, our analysis demonstrated a progressive loss of DNA methylation with increasing maturity in normal B-cells. In an unsupervised approach, we were able to classify bone marrow-derived B-Cells (immature) and peripheral blood-derived B-cells (mature) based on DNA methylation signatures and differentiate both groups from CLL patient genomes, which showed a more pronounced hypomethylation in comparison to normal B-cell groups. A global analysis of histone modification states revealed increased levels of H3K27ac and H3K4me1 in CLL compared to the immature and mature B-cell group genomes, which we could link to the transcriptional up-regulation characteristic of CLL cells.
Conclusion: Utilizing a standardized analytical framework we have generated a comprehensive epigenomic resource that encompasses healthy human subpopulations of maturing B-cells as well as a collection of CLL patient samples. These data has allowed us to begin an in-depth characterization of the epigenetic dysregulation associated with CLL in order to define the mechanisms that drive CLL transformation. Our preliminary findings suggest that the DNA hypomethylation that is associated with normal B-cell maturation is closely tied to the increase in permissive chromatin states that is observed in CLL.

EPI-SEQ: EPIGENOME LANDSCAPE MAPPING FOR CLINICAL AND DRUG DISCOVERY SETTINGS

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Methods for profiling the epigenomic mechanisms of gene regulation have increased in popularity as links between chromatin structure and mechanisms of human disease continue to be discovered. While these assays have become common practice in academic and applied settings, the throughput at which they can be performed and the challenges of interpretation of combined complex datasets has impeded their adoption in clinical settings where large sample numbers are handled by clinical technicians unfamiliar with the technical challenges associated with chromatin immunoprecipitation. Here we describe progress towards the development of Epi-seq, a high throughput multiplexing ChIP-Seq platform to map functional alterations in the epigenome on a scale and time frame suitable for clinical and drug screening applications. With automation compatibility in mind, Epi-Seq combines sample preparation and solubilization of fixed chromatin using PIXUL, a 96-well plate sonicator capable of shearing 96 chromatin samples in less than 30 minutes, with Transposase-Assisted Multiplex ChIP-Seq (TAM-ChIP) to functionally annotate the entire genome in a high throughput format. Multiplexing is achieved with a panel of recombinant AbFlex® ChIP-qualified antibodies targeting various hallmark features of the epigenetic landscape, each of which has been conjugated to a transposase-compatible barcoded oligonucleotide via site-directed conjugation. Optimization of PIXUL conditions using ChIP-seq with antibodies for open (H3K27ac) and closed (H3K27me3) chromatin as a readout will be presented using cell line and tissue derived chromatin, with sonication time and sample requirements as variables. Using four recombinant TAM-enabled AbFlex recombinant antibodies specific for H3K9ac, H3K27ac, H3K36me3, and CTCF, we established concordance between traditional and TAM-ChIP methods and demonstrate the multiplexing capability of TAM-

ChIP using H3K27ac and CTCF AbFlex antibodies. Current and future efforts are focused towards human tissue examples, expansion of the portfolio of ChIP-seq qualified AbFlex recombinant antibodies and expanding the multiplexing capabilities of TAM- ChIP.

ARGONAUTE PROTEINS PROMOTE HISTONE H3K9 TRI-METHYLATION TO INHIBIT TRANSCRIPTION AT DOUBLE STRAND BREAK SITES

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Transcription flanking DNA double strand breaks (DSBs) is known to be suppressed by an ATM mediated pathway to allow rapid repair of DSBs by the non-homologous end joining (NHEJ) pathway. However, transcriptional silencing at DSBs often persists for several hours, well beyond what is required for NHEJ. It is unknown if this prolonged process contributes to or is required for homologous recombination (HR), or if additional mechanisms are required to maintain silencing. On the other hand, several groups have reported that nascent transcripts, as well as small RNAs, arise at DSBs. Therefore, it becomes important to study how transcriptional silencing would coexist with nascent transcription at the DSBs. We hypothesized that Argonaute proteins, likely operating through the damage-induced RNAs (DiRNAs), are responsible for transcriptional silencing at the site of DSBs. Here we show that Argonaute proteins promote local transcriptional silencing by establishing a heterochromatin-like state exhibiting H3K9 tri-methylation and HP1 association. This local H3K9 tri-methylation further stimulates BRCA1 occupancy and DNA end resection at the site of DSBs. We found that transcription initiated local transcriptional silencing at the DSB site is the key event leading to HR. Our results support a model in which a nascent transcript serves as a binding template for Argonaute proteins and DiRNAs, which then direct local H3K9 tri-methylation to retain BRCA1 following induction of DSBs, thereby connecting transcriptional silencing to HR.

PRE-PUBERTAL DIETARY MODULATION ALTERS METHYLATION PATTERN IN BOVINE SPERM

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Enhanced pre-pubertal nutrition in Holstein bulls increased reproductive hormone production (LH, IGF-I, testosterone) and sperm production potential with no negative effects on sperm quality. However, recent trends in human epigenetic research have identified pre-pubertal period to be critical for epigenetic reprogramming in males. Our objective was to evaluate the methylation patterns in the sperm of bulls exposed to different planes of pre-pubertal nutrition. We hypothesised that pre-pubertal nutrition alters methylation patterns in sperm, which is associated with sperm function and early embryo development. Briefly, one-week-old Holstein bull calves, randomly allocated to 3 groups (n=3) were fed either a high-(20% CP, 67.9% TDN), medium-(17% CP, 66.0% TDN) or low-diet (12.2% CP, 62.9% TDN) from 2 to 32 wk of age, followed by medium-nutrition (control). Semen collected from bulls at two specific time points (55-59 and 69-71 wk), was diluted, cryopreserved and used for reduced representation bisulfite sequencing (RRBS). Results demonstrated that diet influenced methylation patterns in sperm. After analysis in DAVID, the GO term, “regulation of Rho protein signal transduction”, implicated in several sperm function attributes (including motility and acrosome reaction) was enriched in both low-vs-high and low-vs-medium datasets ($P<0.1$). Moreover, “MAPK signaling” involved in spermatogenesis and sperm function was upregulated in the 522 genes, differentially methylated in all three diet groups ($P<0.1$). Furthermore, several genes (DNMT3a, INSR, IGF-IR) implicated in early embryo development, including imprinted genes (IGF2, IGF2R) showed differential methylation in response to dietary modulations. Results suggested that pre-pubertal nutrition modulates reproductive potential of dairy bulls through epigenetic mechanisms.

PROFOUND HYPOMETHYLATION OF THE SPERM DNA METHYLOME ASSOCIATED WITH MTHFR DEFICIENCY AND COMPATIBLE WITH FERTILTY

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Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in the one-carbon metabolism pathway with an important role in providing methyl groups for cellular processes including DNA methylation. A common (~12% of the population) human single nucleotide polymorphism, *MTHFR C677T,* is associated with male infertility in some populations. In mouse studies, genetic background influences the degree of infertility found in MTHFR-deficient mice. Here, our aim was to identify the effect of MTHFR deficiency on sperm DNA methylation and to determine whether these changes could be transmitted to offspring. F1 C57BL/6 *Mthfr* $^{-/-}$ male mice were bred with *Mthfr* $^{+/-}$ females to produce F2 generation *Mthfr* $^{-/-}$ sons. Sperm were collected from fathers (F1) and sons (F2) along with wild-type (WT) controls. Sperm DNA was used for genome-wide DNA methylation analysis using Reduced Representation Bisulfite Sequencing (RRBS). We observed 30% lower testis weights in F1 *Mthfr* $^{-/-}$ males compared to WT males; even larger decreases (50%) were seen in F2 *Mthfr* $^{-/-}$ males. As compared to their F1 counterparts, *Mthfr* $^{-/-}$ F2 generation males had higher proportions of abnormal seminiferous tubules and lower fertility rates providing further evidence that reproductive parameters declined between the F1 and F2 generations. RRBS revealed 8359, 100 base pair tiles, to be differentially methylated

in *Mthfr* $^{-/-}$ sperm compared to WT; the majority of the differentially methylated tiles (DMTs) were hypomethylated (8296 tiles) with a small fraction (63 tiles) hypermethylated. A similar trend was observed in F2 *Mthfr* $^{-/-}$ mice with 4198 tiles and 134 tiles, being hypo- and hypermethylated, respectively. Comparing the sperm DNA methylation changes found in MTHFR-deficient males revealed that the magnitude of changes was greater and the size of the affected regions larger in the F1 than the F2 generation. Most strikingly, 81.8% of the hypomethylated F2 DMTs were also found in F1 sperm suggesting that there are regions consistently susceptible to MTHFR deficiency and compatible with fertility.
Further analysis of the characteristics of hypomethylated loci suggested that methylation levels at some regions may be particularly critical for the survival of germ cells, transmission to offspring and the successful development of the next generation through to reproductive age. Together, these results provide evidence that MTHFR deficiency can result in profound hypomethylation of sperm DNA that is compatible with fertility. (Supported by the Canadian Institutes of Health Research)

INTEGRATION OF THE HUMAN PAPILLOMAVIRUS INTO THE HOST GENOME PROMOTES TUMOURIGENESIS BY MODIFYING THE LOCAL EPIGENOME AND TRANSCRIPTOME

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Introduction. Human papillomavirus (HPV) drives almost all cervical cancers and ~70% of head and neck cancers. The HPV double-stranded DNA viral genome integrates into the host genome in >80% of cases. Canonical upregulation of viral oncogenes drives tumourigenesis upon viral integration. We characterized the chromatin structure at HPV integration sites and examined the downstream effects on gene expression.
Methods. We investigated data of 353 HPV-associated cancers from The Cancer Genome Atlas (TCGA) and characterized the epigenome and transcriptome of 5 cancer cell lines with known integrated HPV type 16. We also assessed cell lines by ATAC-seq and CTCF ChIP-seq to evaluate chromatin structure.
Results. We observed unexpectedly low transcription of E6 and E7 in a group of HPV^{+} patients. We hypothesized that changes in the epigenome and transcriptome contribute to tumorigenesis of HPV alongside HPV oncogenes. We found striking evidence for a dramatic change in local chromatin structure and gene expression in the vicinity of viral integration events. We found that a conserved CTCF sequence motif within the HPV genome binds CTCF in cell lines. In concert with these local changes in CTCF binding, we found consistent increase in chromatin accessibility of cell lines within a 100 kbp window of HPV integration sites. Changes in the chromatin landscape occurred concomitant with outlier expression and alternative splicing of genes at the vicinity of HPV integration sites. Some of these genes such as *FOXA1*, *SOX2*, *E2F1*, and *PBX1* among others have well-known roles in tumour development and progression.
Conclusion. Our results suggest that CTCF binding to the HPV genome reorganizes the chromatin and modifies the transcriptome in HPV-associated cancers. We consistently observed altered epigenome and transcriptome in cell lines and patients with matched RNA-seq and ATAC-seq data. These findings indicate that HPV integration *per se* contributes to tumour development.

HISTONE H3.3 G34W MUTATION IN NEOPLASTIC STROMAL CELLS PROMOTES ECM REMODELING AND DRIVES TUMORIGENESIS OF GIANT CELL TUMOR OF BONE

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In several cancer types, somatic mutations occur at specific residues of histone H3 genes. Intriguingly, mutations at the glycine 34 (G34) residue in *H3F3A* encoding H3.3 occur in two cancer types: giant cell tumors of bone (GCTB) where G34 is mutated to either tryptophan (G34W) or leucine (G34L), while in pediatric high-grade glioma (pHGGs) G34 is mutated to arginine (G34R) or valine (G34V). GCTB are locally aggressive tumors which contain three histological cell types: multinucleated, osteoclast-like giant cells; monocytic, macrophage-like cells; and mesenchymal stromal cells. The stromal cells are the only compartment that carry the H3.3 G34W mutation.
To study the H3.3G34W mutation in neoplastic stromal cells, we generated patient-derived isogenic models using CRISPR/Cas9 to correct the *H3F3A* G34W mutation to wildtype (edited) in stromal cells derived from GCTB tumors.
Correction of the H3.3G34W mutation in edited lines significantly reduced cell proliferation and colony forming potential *in vitro*. We also observed morphological differences between G34W and edited lines. Importantly, orthotopic tibial and subcutaneous injection of edited stromal cells did not yield tumors in immunodeficient mice, while injection of H3.3G34W stromal cells resulted in the formation of aggressive and osteolytic orthotopic tumors and recruitment of H3.3G34W-negative osteoclast giant cells.
We then performed mass spectrometry of proteins found within the Golgi apparatus of isogenic GCTB stromal cells to identify the secreted factors by H3.3G34W stromal cells responsible for recruiting osteoclasts into the tumor. Differentially secreted factors between H3.3G34W and edited cells included several cell adhesion proteins (e.g. collagens) secreted into the extracellular matrix (ECM) by H3.3G34W cells.
Cell morphological, functional and tumorigenic differences suggested a transformation in cell identity upon correction of the H3.3G34W mutation in stromal cells. We attempted to characterize the cell of origin of GCTB stromal cells and

H3.3G34W-mediated alteration of stromal cell identity. Previous reports imply that GCTB stromal cells derive from premature osteoblasts and can be induced to differentiate to osteoblasts *in vitro*. Strikingly, the isogenic GCTB stromal cells (H3.3G34W and edited) failed to differentiate to a number of mesenchymal lineages (osteoblastic and adipocytic) but could be induced to differentiate to smooth muscle regardless of their genotype.
Altogether, we propose that GCTB H3.3G34W stromal cells represent a mesenchymal cell type of indeterminate classification with functions relevant to ECM remodeling and development of a microenvironment that promotes recruitment of resorptive multinucleated giant cells, the defining pathological feature of GCTB. Moreover, our findings underscore an enduring role for the H3.3G34W oncohistone mutation in GCTB tumorigenesis.

A MULTI-STAGE EPIGENOME-WIDE ANALYSIS REVEALS OBESITY-SPECIFIC NOVEL CPG SITE IN KOREAN

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We performed an epigenome-wide analysis of multiple stages to find significantly different DNA methylation site between obese and lean participants. Genome-wide methylation levels in whole blood were measured using HumanMethylationEPIC array (phase I, n = 450) with replication in an independent group (phase II, n = 377) from different population-based cohorts. Additional three different datasets - EPIC arrays of blood and fat, and HT-12 Expression BeadChip for fat tissue - were utilized for replication from the hospital-based participants (phase III, n = 75). Significant CpG sites were also examined in HumanMethylation450K array from childhood obesity subjects (n = 94) and RNA sequencing data from fat-isolated cells (adipocytes and preadipocytes, n = 26 and 21 each) were used for assessment of gene expression difference on the identified obesity-associated genes. 142 obesity-specific differentially methylated positions (DMPs) in discovery (false discovery rate (FDR) < 0.05) and replication ($p < 0.05$), and corresponding annotated genes were associated with diseases and functions mainly regarding lipid metabolism. Among them, 48 sites exhibited unidirectional changes in methylation differences through stages and comprised 24 sites with methylation-expression correlations (|spearman's r| > 0.2) in adipose tissue. Taking into account the multiple evidences with obesity-specificity of the prioritized cg13424229 annotated to CPA3, DNA methylation in combination at gene expression level could provide functional insights in the pathogenic mechanisms of human obesity.

KOREA, KNIH EPIGENOME PROJECT 2019

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Korea National Institute of Health (KNIH) as an official member of IHEC launched the KEP (Korea Epigenome Project) in 2012 with the aim of activation of epigenomics research in Korea. The project is subject to produce 50+ reference epigenome datasets on Korean chronic disease (diabetes, obesity, chronic kidney disease, etc.) related cells with the participation on IHEC. The project has been producing IHEC's core datasets with DNA methylation, RNA expression, and ChIP-seq of 6 histone modifications for unravelling epigenomic differences among cell-types of diseases. As of the end of 2019, 57 epigenome datasets will be released through European Genome Archive (EGA) and data portals of IHEC and KEP with informative metadata. The Researchers interested in these KEP data have been applied through KEP°Øs data access committee on the EGA and utilized Korean reference epigenome datasets. Processed epigenome datasets in BigWig format have been accessed both in data portal of IHEC and KEP for thousand times worldwide. Since 2017, KNIH and the epigenome research groups of Korean scientists had been organized and utilized datasets targeted for Korean chronic diseases in terms of disease-specificity and analytical techniques, and groups have reported epigenetic specificities of the diseases (RA and Obesity). In the following years, our previously published datasets will be merged into and systematically managed at CODA (Clinical and Omics Data Archive) in KNIH, and additional epigenome datasets are planned. Disease specifications/categories for Korean epigenome datasets will be further discussed in detail.

LEARNING A GENOME-WIDE SCORE OF EVIDENCE FOR CONSERVATION IN HUMAN AND MOUSE FROM LARGE-SCALE FUNCTIONAL GENOMIC ANNOTATIONS

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Comparing epigenomic data between human and mouse can be useful in characterizing DNA elements in both species and cross-species variations. There is a growing collection of functional genomic annotations from numerous human and mouse cell-types such as maps of open chromatin and histone modifications. Previous methods have focused on one type of annotation at a time when comparing the two species. To take better advantage of the available data, here we develop a computational method that integrates thousands of functional genomic annotations to learn a score of evidence for conservation at the functional genomics level without explicitly matching the annotations by their biological origin and data type.
The annotations we use as features consist of ChromHMM chromatin state annotations, signals from RNA-seq experiments, and peak calls from DNase-seq, transcription factor ChIP-seq, histone mark ChIP-seq, and CAGE experiments. Given a human region and a mouse region, we generate a pair of feature vectors, one corresponding to features annotating the human region and

the other corresponding to features annotating the mouse region. We train an ensemble of neural networks that take pairs of feature vectors from human and mouse regions aligning to each other at the sequence level as positive samples and pairs of feature vectors from randomly matched human and mouse regions that do not align to each other but somewhere else in the other species as negative samples. We apply the trained classifier to the genomes to score all pairs of aligning regions with the human-mouse Functional Genomics Conservation (FGC) score.
The FGC score differentiates aligning pairs from randomly matched non-aligning pairs with an area under the ROC curve of 0.86. We validate that high-scoring regions tend to have DNase-seq or H3K27ac ChIP-seq peaks in similar sets of tissues. DHS, CpG islands, and transcription start sites tend to score highly. The score provides complementary information to sequence constraint annotations, prioritizing potentially important genomic regions with little sequence constraint but annotated with largely overlapping sets of chromatin states in human and mouse. High-scoring regions are enriched for phenotype-associated variants and partitioned heritability of complex traits. Applying the FGC score to an epigenetic study in human and mouse revealed that the score has predictive power for genomic regions with conserved differential methylation patterns in diabetic phenotypes. The FGC score could contribute to a broad set of applications, ranging from prioritizing human variants to test in mouse to analyzing cross-species epigenetic studies.

SINGLE-CELL TRANSCRIPTOMES REVEAL A MYC-DRIVEN REPROGRAMING OF THE ANDROGEN RECEPTOR CISTROME IN PROSTATE CANCER

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Prostate cancer (PCa) is the most prevalent cancer in men and a leading cause of cancer-related lethality. PCa development involves corruption of the normal prostate transcriptional network, following deregulated expression or mutation of key transcription factors. MYC is a major driver of PCa tumorigenesis and progression. Although MYC is overexpressed in both early and metastatic disease and associated with poor survival, its impact on prostate transcriptional reprogramming remain elusive. Using a MYC-driven genetically engineered mouse model of PCa that largely mimics the human disease, we have generated the transcriptome profiling at a single-cell level of both normal and MYC-transformed murine prostate lobes. Here, we demonstrate that MYC overexpression significantly diminish the androgen receptor (AR) transcriptional program in luminal prostate cells without altering its expression. Covariance analysis revealed that upon MYC overexpression, transcripts co-expressed with AR shift from canonical targets to transcripts related to ribosome biogenesis. Importantly, chromatin immunoprecipitation followed by sequencing of the AR uncovered a profound reprogramming of its cistrome following MYC overexpression. Data integration with single cell transcriptomics further revealed the establishment of a corrupted AR transcriptional program redirected toward MYC targets. Our findings suggest that in primary prostate cancer, MYC overexpression contributes to tumour initiation and progression by hijacking the AR transcriptional program.

THE ROLES OF NUA4/TIP60 AND SAGA ACETYLTRANSFERASE COMPLEXES IN THE REPAIR OF DNA BREAKS THROUGH DIFFERENT PATHWAYS

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Among the different types of DNA lesions, double-strand breaks (DSBs) are the most deleterious type if not precisely repaired. Regulation of the chromatin state is a crucial step for proper repair of the damaged DNA, maintenance of genome stability and cellular fitness. Histone post-translational modifications, incorporation of histone variants, and DNA methylation can dynamically regulate the chromatin structure and DNA accessibility, providing docking sites for the recruitment of repair machineries. Specific chromatin modifications can establish a chromatin setting permissive for different DSB repair pathways. DSBs are repaired by non-homologous DNA end-joining (NHEJ) throughout the cell cycle and homologous recombination (HR), alternative end-joining (alt-EJ), and single-strand annealing (SSA) in S and G2 phases. Here we investigated the role of the SAGA and NuA4/TIP60 acetyltransferase complexes in the DNA repair pathway choice. SAGA is composed of 19 subunits organized in different functional modules. The SAGA histone acetyltransferase module (HATm) contains PCAF or GCN5 as catalytic subunits. Its deubiquitination module (DUBm) contains the USP22 DUB enzyme. Our study revealed the existence of a DSB-induced monoubiquitination-to-acetylation switch on histone H2BK120 around DNA breaks, most likely mediated by the SAGA complex. Consistently, we demonstrated that the native human SAGA complex displays both H2BK120 deubiquitinase and acetyltransferase activities for this site *in vitro*. We showed that the depletion of SUPT7L, PCAF, and USP22 leads to decreases in both HR and NHEJ using cell reporter systems. We further showed that while the depletion of different subunits of NuA4/TIP60 decreases the efficiency of HR and SSA, the depletion of SAGA HATm promotes SSA. Notably, USP22 depletion resulted in a decrease in SSA. We also found that the native human SAGA and Tip60 complexes acetylate RAD52 *in vitro*. These results suggest that the depletion of SAGA HATm may create conditions that would favor SSA through either

promoting resection in an unscheduled way and/or acetylation of DNA repair factors. Taken together, our results suggest that HATm and the DUBm of the SAGA may play opposite roles in the DNA repair process such as the establishment of a chromatin state competent for DNA end resection and/or synapsis to prevent unscheduled SSA repair pathway.

CELL-FREE FETAL DNA METHYLATION PROFILES OF MATERNAL OBESITY EFFECTS ON INFANT NEURODEVELOPMENT IN NON-HUMAN PRIMATES

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Maternal obesity increases the risk for autism spectrum disorders (ASDs) but not all pregnant women who are obese are at risk for having a child with an ASD. As interventions for ASDs are most effective when implemented early, modern diagnostics for associated prenatal exposures are needed. During pregnancy, placental DNA, which is of fetal origin, is released into the maternal bloodstream as cell free fetal DNA (cffDNA) and these DNA methylation profiles can be examined non-invasively from maternal blood samples. The placental methylome contains large-scale partially-methylated domains (PMDs), which function as chromatin "storage boxes" for neurodevelopmental genes, where early developmental exposures are reflected as methylation differences. This study leverages naturally obese rhesus macaques that give birth to offspring with altered behavior. In our study of 25 rhesus macaques pregnant with male fetuses, lean control dams were matched to obese dams distributed into 3 groups, including untreated, calorie restricted, and pravastatin treated. Whole genome bisulfite sequencing (WGBS) was utilized to assay cffDNA collected from maternal blood at four gestational time points (n=96). The cffDNA methylomes were aligned using the CpG_Me pipeline and statistically analyzed with the DMRichR package. Strikingly, our preliminary results show that cffDNA methylation profiles of >10 million CpGs can distinguish obesity exposed fetuses from matched controls in the first trimester of pregnancy. We identified >4000 significant differentially methylated regions (permutation p<0.05) that represent significant enrichments (p<0.05) for genes involved in neurodevelopment, metabolism, and the regulation of gene expression. Furthermore, these profiles are present in the later trimesters of pregnancy and are modified in a complex manner by the dietary and pharmacological obesity interventions. Ongoing efforts are focused on the sequencing of matched 6-month-old brain samples and the integration of the analyses with infant behavioral analyses through weighted gene co-methylation network analysis (WGCNA) and machine learning approaches. This work is the first to generate epigenetic profiles of both placental and brain DNA methylation in a non-human primate model of obesity. These findings may be translated not only to identify the risk for ASDs and neurodevelopmental disorders in human pregnancies, but to also guide behavioral and therapeutic interventions in perinatal health care.

UNDERSTANDING CTCF DYSFUNCTIONS IN CANCER

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Background: Tumor suppressor proteins act to limit the proliferation and survival of cells, thereby preventing cancer and its spread to throughout the body. CTCF is a protein whose normal function is commonly disrupted in a wide array of cancer, including loss of heterozygosity in 50% of breast cancers. Substantial evidence demonstrates that CTCF acts as a tumor suppressor gene[1-3]. However, the role of CTCF deletion or mutation in cancer has not been clearly elucidated. Our novel investigation will uncover the mechanism of action of CTCF in cancer progression by studying CTCF single allele deletion and the most common missense mutations found in human tumors, starting with CTCF H284N, which is found enrich in breast tumors resisting hormone therapy[4].

Aim #1: To examine an oncogenic role for CTCF LOH. As a model for CTCF LOH, we used CRISPR/Cas9 to knockdown WT CTCF from a single allele in a non-transformed breast epithelial cell line, MCF10A. CTCF +/- cells showed increased invasiveness and mammosphere growth *in vitro*. This translated to an ability to initiate tumors at incomplete penetrance and increased metastatic abilities *in vivo*. RNA-seq indicated the classical oncogene Snail was strongly upregulated in CTCF+/- cells and that the PI3K pathway might be also activated, which was subsequently validated. ChIP-Seq revealed that CTCF binding was lost around key PI3K genes and also from the Snail promoter. Based on this, we tested the sensitivity of CTCF +/- cells to inhibition of the downstream PI3K effector, mTor, using Torin1. As expected, the invasion of CTCF+/- cells were highly sensitive to Torin1 or Snail knockdown. At the epigenetic level, CTCF loss leads to aberrant accumulation of H3K4me3 at key oncogenes promoting their upregulation. We are currently examining the changes in 3D chromatin structure surrounding PI3K genes and Snail using Hi-C.

Aim #2: To define the oncogenic impact of CTCF mutations. As a model for CTCF H284N mutation, we again employed CRISPR/Cas9 to conduct a biallelic knock-in of the H284N mutation in MCF10A cells. We screened for oncogenic phenotypes and found that the CTCF mutation potentiate invasiveness *in cellulo*. Surprisingly, H284N mutation synergizes with MAPK pathway activation to promote cell invasion. CHIP-Seq on CTCF binding revealed the unexpected result that CTCF mutation modulates CTCF binding to not only lose association, but unexpectedly, to gain an equivalent number of sites. Analysis of these altered binding sites, and RNA-seq experiments, revealed that altered CTCF binding leads to changes in transcriptional networks involved in metabolism and cell adhesion, perhaps explaining why CTCF mutation is enriched in drug resistant tumors. We are currently examining the impact of CTCF mutation on 3D chromatin structure.

Overall, these studies reveal mechanistically how CTCF dysfunction drives oncogenic progression and provides insight

into therapeutic avenues which might be used to target tumors carrying these molecular phenotypes.

A SYSTEMATIC ANALYSIS OF CELL-TYPE SPECIFICITY WITH GENE EXPRESSION PATTERNS AND EFFECTS OF EPIGENOMIC REGULATION

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The human body consists of more than 200 types of cells in various tissues that have own functions with different biological processes, and this tissue/cell-specific processes in human may results in various diseases. According to numerous research, the patterns of gene expression are tissue-specific and highly affected by epigenetic modifications, such as DNA methylation and histone modification. Here, we identified tissue specific genes (TSGs) and confirmed that those genes were whether positively or negatively controlled by DNA methylation on their promoter region. First of all, we calculated tissue-specificity metric using 52 datasets from five different human cell types (adipocytes, islets, kidney cells, skeletal muscle cells and fibroblasts). Secondly, we examined the promoter-wide DNA methylation patterns with correlation analysis between gene expression levels (TPM) and DNA methylation levels (mean of beta value) on promoter region of TSGs. As a result, we found 320 of negatively- and 113 positively correlated pairs of TSGs, of which the majorities were related to cardiometabolic diseases, in particular. Our results of cell-type specific and epigenetically regulated gene expression may provide a clue that epigenetic changes with tissue specific pattern can help understanding diseases mechanisms.

ROLES OF PERICONCEPTIONAL MODERATE AND HIGH DOSE FOLIC ACID SUPPLEMENTATION IN THE PREVENTION OF EPIGENETIC DEFECTS ASSOCIATED WITH THE USE OF ASSISTED REPRODUCTION

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Assisted reproductive technology (ART), used to assist infertile couples conceive children, currently accounts for 1-4% of all births in Western countries. Increases in growth and birth defects, accompanied by DNA methylation abnormalities, have been reported in ART-conceived children. Proper DNA methylation is crucial for normal development of the embryo and placenta. Dietary folic acid is a major source of methyl groups used in DNA methylation. Here, our goal was to use a mouse model to determine how ART, including superovulation, in vitro fertilization, embryo culture and transfer, and different folic acid supplemented diets (control diet, 4 times control diet, and 10 times control diet) could affect DNA methylation of selected non-imprinted and imprinted genes in placentas from morphologically normal but growth retarded conceptuses. Bisulfite pyrosequencing was used to look more closely at DNA methylation of genes previously shown to be affected by ART in growth retarded embryos by genome-wide analysis. The placenta was used here as it has been shown to be more susceptible than the embryo to the induction of epigenetic defects. For *Casz1*, a non-imprinted gene, DNA methylation was not affected by ART or the different doses of folic acid. In contrast, although the mean DNA methylation of the germ line differentially methylated region (gDMR) of *Nnat*, an imprinted gene, was not affected by ART or folic acid supplementation, ART resulted in a significant increase in variance of *Nnat* methylation with evidence of correction of the ART effect with both doses of folic acid. Even more dramatic findings were evident for the imprinted gene *Gnas*, also one of the genes most highly affected in the growth retarded embryos. ART resulted in a decrease in mean methylation and an increase in variance for different regions of *Gnas*; partial correction of the ART effect occurred following low dose but not high dose folic acid treatment. The results suggest that non-imprinted and imprinted genes are differentially affected in the placentas versus the embryos of ART conceived growth retarded conceptuses and that folic acid supplements can partially correct ART induced DNA methylation defects in a dose-dependent manner.

EVOLUTION OF IMPRINTING VIA LINEAGE-SPECIFIC INSERTION OF RETROVIRAL PROMOTERS

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Imprinted genes (IGs) are monoallelically transcribed, with paternally (*Pegs*) and maternally expressed genes (*Megs*) almost equally represented in the mouse genome. Most imprinted expression stems from DNA methylation (DNAme) marks differentially inherited from the gametes and maintained throughout development. In the mouse, most DNAme imprints are of maternal origin and are established postnatally in oocytes. Interestingly, *de novo* DNAme occurs within transcribed regions in oocytes. Consequently, most imprints at *Pegs* mark their CpG island (CGI) promoters, acquiring DNAme because they are covered by an oocyte-specific transcript.
Here we show that transcription initiating in proximal lineage-specific endogenous retroviruses (ERVs) is likely responsible for DNAme established in oocytes at 4/6 mouse-specific and 17/110 human-specific maternal imprinted gDMRs (igDMRs). The latter can be further divided into Catarrhini (Old World monkeys and apes)- or Hominoidea (ape)-specific igDMRs, which are embedded within transcription units initiating in ERVs specific to these primate lineages. Using CRISPR-Cas9 mutagenesis, we deleted the relevant murine-specific ERVs upstream of the maternally methylated genes *Impact* and *Slc38a4*. Strikingly, imprinting at these genes was lost in the offspring of females harboring these deletions and biallelic expression was observed.

Our work reveals a novel evolutionary mechanism whereby maternally silenced genes arise from biallelically expressed progenitors.

DEVELOPMENTAL GENOME-WIDE DNA METHYLATION ASYMMETRY BETWEEN MOUSE PLACENTA AND EMBRYO

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In early embryos, DNA methylation is remodelled to initiate the developmental program but for mostly unknown reasons, methylation marks are acquired unequally between embryonic and placental cells. To better understand this, we generated high-resolution DNA methylation maps of mouse mid-gestation (E10.5) embryo and placenta. We uncovered specific subtypes of differentially methylated regions (DMRs) that contribute directly to the developmental asymmetry existing between mid-gestation embryonic and placental DNA methylation patterns. We show that the asymmetry occurs rapidly during the acquisition of marks in the post-implanted conceptus (E3.5-E6.5), and that these patterns are long-lasting across subtypes of DMRs throughout prenatal development and in somatic tissues. We reveal that at the peri-implantation stages, the *de novo* methyltransferase activity of DNMT3B is the main driver of methylation marks on asymmetric DMRs, and that DNMT3B can largely compensate for lack of DNMT3A in the epiblast and extraembryonic ectoderm, whereas DNMT3A can only partially palliate in the absence of DNMT3B. However, as development progresses and as DNMT3A becomes the principal *de novo* methyltransferase, the compensatory DNA methylation mechanism of DNMT3B on DMRs becomes less effective.

G9A REGULATES DNA METHYLATION AND CHROMATIN LOOPING IN DISTINCT MECHANISMS

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The lysine methyltransferase G9a is responsible for depositing Histone 3 lysine 9 (H3K9) methylation, which is associated with transcriptional repression. Previous works have demonstrated that the loss of G9a disrupts the epigenome and potentially the chromatin structures of murine pre-implantation embryos. Yet the mechanisms remain unclear. To delineate the molecular function of this protein, we conducted an in-depth integrative analysis of G9a depleted and G9a catalytic mutant embryonic stem cells (mESCs). We found that the catalytic activity of G9a was required for maintaining genome-wide DNA methylation. However, the hypomethylation in these mutant cells was not restricted to H3K9 dimethylation (H3K9me2) marked regions. Further investigation on chromatin accessibility and epigenomic modifications demonstrate that G9a functions in both catalytic dependent and independent manners. Strikingly, dysregulated retrotransposons were shown to serve as non-canonical promoters or contribute to alternate splicing events, resulting in transcriptomic changes. Moreover, we also discovered that while topological associated domains (TADs) and compartments A/B definitions remain largely unaffected, chromatin loops formation at TAD boundaries were weakened in G9a mutant cells. The phenotype was dependent on G9a-mediated H3K9me2 but independent of DNA methylation. Taken together, our findings enhanced the understanding of the role of G9a in regulating the epigenome, transcriptome and chromatin organization of mESCs.

A CELL TYPE-AGNOSTIC REPRESENTATION OF THE HUMAN EPIGENOME THROUGH A DEEP RECURRENT NEURAL NETWORK MODEL

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Sequencing-based assays such as ChIP-seq and ATAC-seq have recently been used to characterize the the epigenome of hundreds of human cell types. These data sets necessitate the integrative methods to summarize them into a useful representation. A popular type of existing method is segmentation and genome annotation (SAGA) algorithms such as Segway and ChromHMM, which produce an annotation of the epigenome of a given cell type. However, annotations are cell type-specific. Therefore, they cannot easily be used to understand the function of a locus in the context of phenotype as a whole. Therefore, we need a cell type-agnostic view of the epigenome that is analogous to existing annotations of protein-coding genes. Existing SAGA algorithms use simple discrete or linear models, which cannot capture the complexity of the epigenome across all cell types.
We propose a method that reduces all existing epigenome data into a single low-dimensional representation. This representation assigns a vector of features to each genomic position that captures that position's activity across all tissues. We do this using a deep long short-term memory (LSTM) recurrent neural network model. In contrast to existing neural network models of the genome, this model is sequential and therefore captures the spatial relationships of neighboring genomic positions.
We demonstrate the utility of this representation through several analyses. First, we show that original data can be accurately reproduced using just this latent representation, indicating that little information is lost in this reduction. Second, we show that this representation simultaneously captures many types of cell type-specific activity across many cell types, including gene expression, replicating timing and chromatin contacts. We do this by demonstrating that all of the above phenomena are accurately predictable using just the latent representation. Third, we show

that this latent representation distinguishes functional and non-functional regions by showing that the representation accurately identifies conserved regions and disease-associated loci. Fourth, we demonstrate how a sequential model leads to a more interpretable representation than existing methods, which do not capture dependence among neighboring positions.
Thus, we expect that this latent representation will be widely used for many genomic analyses, including identifying novel genomic elements and understanding the function of disease-associated variants. This latent representation forms a succinct, cell type-agnostic representation of epigenetic function and therefore greatly simplifies these tasks. In addition, by reducing the size of epigenetic data by nearly a factor of 100, the resulting representation makes downstream tasks much more computationally manageable.

PATERNAL FOLATE DEFICIENCY INDUCES ABERRANT HISTONE METHYLATION IN SPERM WHICH IS TRANSMITTED TO THE PRE-IMPLANTATION EMBRYO

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BACKGROUND. Every year, birth defects affect 7.9 million infants worldwide. Although some birth defects are caused by genetic factors or infectious diseases, over 50% of them remain idiopathic (WHO, 2016). Paternal exposures to poor nutrition lead to metabolic changes and increased incidence of birth defects in the offspring (Lambrot et al., 2013, Radford et al., 2014). These inherited phenotypes have been attributed to altered epigenetic information in sperm yet the mechanisms remain poorly defined. In this study, we elucidate how a paternal folate deficiency perturbs the sperm epigenome, alters the pre-implantation embryo chromatin landscape, and impacts offspring development.
METHODS. To determine the effects of a folate deficient diet on the sperm epigenome, we fed a folate sufficient (FS, 2.0 mg/kg) or folate deficient (FD, 0.3 mg/kg) diet to wildtype males for two full spermatogenic cycles and performed ChIP-Seq for histone 3 methylation* on their sperm. We used Ultra-Low-Input ChIP-Seq on 8-cell embryos from FS or FD wildtype males to identify regions with paternally-inherited H3me alterations. To assess whether multiple epigenetic stressors could cumulatively erode sperm H3me levels, we employed ChIP-Seq for H3me on the sperm of FS and FD transgenic males that overexpress the histone demethylase KDM1A in their germ cells (Siklenka et al., 2015). We evaluated skeletal defects in E18.5 fetuses from wildtype or transgenic males fed a FS or FD diet to determine if the combination of a folate deficiency and KDM1A overexpression enhanced developmental defects in the offspring. Finally, low-input RNA-Seq of 8-cell embryos from FS or FD males on a wildtype or transgenic background allowed us to assess how diet-induced H3me alterations in sperm impacted embryonic gene expression.
RESULTS. We identified 1434 regions with altered H3me in the sperm of FD wildtype males. These regions intersected critical developmental promoters, enhancers and transposable elements. Gene ontology analysis revealed that affected pathways were involved in morphogenesis, bone development, and chromatin remodeling. Strikingly, aberrant H3me patterns in FD wildtype sperm were retained in the pre-implantation embryo. We further demonstrated that feeding a FD diet to KDM1A transgenic males further exacerbated sperm H3me enrichment defects. E18.5 skeletal analysis of fetuses from FD transgenic males revealed a significant increase in severe abnormalities in the offspring. These abnormalities included craniofacial and spinal defects as well as pronounced developmental delays. Low-input RNA-Seq analysis of 8-cell embryos is ongoing.
CONCLUSION. Our study demonstrates that sperm H3me serves as a key determinant in transmitting environmentally-induced phenotypes to the pre-implantation embryo, and contributes to the broader understanding of how paternal lifestyles can influence embryonic development and offspring health.
*patent pending

ATAC-SEQ BASED FOOTPRINTING ANALYSIS FROM HUMAN PREIMPLANTATION STAGES: WHAT CAN WE LEARN ON TFS ACTIVITY FROM CHROMATIN ACCESSIBILITY?

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Transcription factors (TFs) play important roles in regulating epigenetic processes throughout preimplantation development. While ChIP-based methods for investigating protein binding are generally unfeasible in the context of low-input samples such as embryo, ATAC-seq can be performed with very low input. In this context, digital genomic footprinting (DGF) analysis has shown great potential to predict transcription factor binding sites (TFBS) from the profiles of chromatin accessibility. **Similar to nucleosomes, DNA-binding proteins such as TFs bound to open chromatin regions hinder cleavage, resulting in regions of decreased signal strength within regions of high signals - known as *footprints***. This concept shows great potential, as it enables researchers to investigate multiple TFs at a time using just a single experimental assay. However, the lack of an established framework for various steps of footprinting analysis has hindered the widespread application of this method. **Here we describe the application of a new software called TOBIAS** (**T**ranscription factor **O**ccupancy prediction **B**y **I**nvestigation of **A**TAC-seq **S**ignal), a comprehensive computational framework enabling start to end digital genomic footprinting analysis on a genome-wide scale. TOBIAS provides individual modules for all steps of the analysis including bias-correction, footprinting and visualization, as well as complete analysis workflows. We exemplarily use this tool to study the changes in TF binding across preimplantation development integrating in vitro and in vivo datasets from human and mouse. By utilizing the TOBIAS

network and target prediction module to a key TF of preimplantation (Dux), we further utilize footprints to predict transcription factor targets and transcription factor activation cascades. We thereby provide a proof-of-concept for digital genomic footprinting as a versatile tool to gain understanding of transcription factor binding dynamics.

THE EFFECT OF TAD BORDER DISRUPTION AT THE *PDGFRA/KIT/KDR* LOCUS ON 3D CHROMATIN STRUCTURE AND GENE EXPRESSION

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Genomes of higher eukaryotes are partitioned into sub-megabase self-interacting units called topologically associating domains (TADs), which are widely conserved in mammals. CTCF, cohesin and condensin complexes are proven to be the pivotal players in TAD formation. It has been demonstrated that dislocations in TAD border regions and disruption of CTCF-binding sites lead to dramatic changes in gene expression patterns, depletion of TADs, and, in some cases, activation of oncogenes. However, according to the latest reports, there are multiple examples where removing the TAD boundary does not cause any detectable consequences. Apparently, for different loci, the functional role of TADs might not be as straightforward as it has been considered, and new studies at various genomic regions can help to uncover fundamental links between the 3D chromatin structure and gene regulation.
For our study, we selected the locus harboring three genes (*Pdgfra/Kit/Kdr*) all of which encode tyrosine kinase receptors that are important for differentiation and determination of particular cell types, such as fibroblasts, mast cells, and endotheliocytes. Each experimental gene belongs to a separate TAD. Using CRISPR/Cas9 editing system, we generated a set of five mouse lines carrying deletions of CTCF sites, which determine the formation of TAD borders at this locus. In the case of *Kit/Kdr* border deletion, we observed that the TADs did not merge but the new border seems to appear at the nearest CTCF site. This could be an evidence of alternative mechanisms involved in maintaining the TAD structure and insulating the gene neighborhood. At the organism level, we have shown that removing the boundaries of TADs does not lead to developmental abnormalities or any phenotypic manifestations. However, we found that a large 300 kb deletion, which removes *Kit* gene and most of its TAD, causes white-spotted phenotype in mice and, strikingly, leads to a mast cell depletion even in heterozygous animals. The molecular mechanism of the mast cell depletion remains to be explored. *Kit* is known to be one of the most active genes in mast cells, which transcription is regulated by the group of active enhancers. Therefore, we tested the hypothesis whether the removal of CTCF sites at the TAD border would lead to incorrect activation of neighboring genes. Remarkably, mast cell RNA-seq analysis did not reveal any significant alterations in expression levels of surrounding genes. Thus, the removal of TAD boundaries at the *Pdgfra/Kit/Kdr* locus does not affect gene expression.
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ALLELE-SPECIFIC CHROMATIN CONFORMATIONS IN TROPHOBLAST STEM CELLS REVEAL ACTIVATING AND REPRESSING REGIONS FOR SFMBT2, AN IMPRINTED POLYCOMB-GROUP GENE

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Sfmbt2 is a maternally imprinted gene encoding a polycomb-group protein critical for extraembryonic tissue development and derivation of trophoblast stem cells (TSC) in *Mus musculus*. Lack of paternal *Sfmbt2* results in reduced placental size leading to pre-term mortality in mice. Paternal-specific H3K27ac and H3K4me1 peaks along with maternal-specific H3K27me3 peaks at the *Sfmbt2* promoter proximal region reflect the paternal-specific expression patterns of *Sfmbt2* in TSCs. Although *Sfmbt2* is highly expressed in TSCs, cell-type specific regulation of Sfmbt2 transcription remains poorly understood.
We performed a genome-wide enhanced 4C-seq (e4C) to screen for looping events with the Sfmbt2 promoter to identify novel distal regulatory elements controlling *Sfmbt2* expression in TSCs. The paternal *Sfmbt2* promoter preferentially interacts with a contact region 60kb upstream that we classified as a putative enhancer due to enrichment in H3K27ac and H3K4me1. This region contains putative binding sites for TSC transcription factors Elf5, Eomes and Cdx2. Allele-specific 3C (AS3C) confirmed this putative enhancer region interacts preferentially with the paternal promoter. Reporter gene assays confirm that this region interacts specifically with the *Sfmbt2* promoter to act as an enhancer. Cdx2, a transcription factor required for trophoblast lineage, binds the enhancer indicating that this region could regulate target genes through a Cdx2 dependent mechanism.
A proximal region 250kb downstream of *Sfmbt2* interacts more frequently with the *Sfmbt2* promoter on the silenced maternal allele. The downstream region is characterized by repressive H3K27me3 marks and lacks H3K27ac and H3K4me1 signals.
Distal to *Sfmbt2*, interactions between *Sfmbt2* promoter and euchromatic domains were enriched on the paternal allele whereas the maternal *Sfmbt2* promoter was associated with heterochromatic domains in TSCs. This combination of e4C, AS3C and chromatin analysis indicates that the paternal *Sfmbt2* promoter is associated with a euchromatin compartment and loops out to upstream enhancer regions whereas the maternal *Sfmbt2* promoter is compacted and interacts with the proximal downstream H3K27me3 region.

CROSS-STUDY HARMONIZATION OF ALL AVAILABLE RAW HM450K METHYLATION ARRAY DATA FROM THE GENE EXPRESSION OMNIBUS WITH RECOUNT-METHYLATION

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DNA methylation (DNAm) is the most-studied epigenetic mark in primary samples. The platform of choice for large-scale profiling is the Illumina methylation arrays. This platform has been widely used to interrogate normal tissue development, gene regulation dynamics, gene-environment interactions, and disease risk and progression. While hundreds of studies have published tens of thousands of DNAm array samples on the Gene Expression Omnibus (GEO), no resource harmonizes sample DNAm and metadata across all studies, and it remains difficult for researchers to identify, aggregate, and analyze this wealth of data.

We performed what is, to our knowledge, the largest cross-study analysis of DNAm arrays. For this, we obtained and harmonized sample metadata and raw HM450k IDAT files from approximately 400 studies and 35,000 samples on GEO. We characterized DNAm differences across non-cancer tissues, compared epigenetic age to chronologic age, and investigated Houseman cell type deconvolutions across samples. We further performed thorough cross-study quality control analyses, including summaries of Illumina control probe metrics, evaluation of control probe signal failure rates, batch effect quantification and correction, and assessment of the relationships between probe detection p-values, signal levels, and tissue types.

In tandem with our cross-study analysis, we are preparing the first version of the recount-methylation resource for open-access release. This resource will make harmonized sample and DNAm data readily accessible and analyzable by researchers. Harmonized sample data will include study metadata, annotations of sample type and origin, machine learning-mapped ontology terms, and DNAm-based predictions of age, sex, and cell type abundances. After initial release, we intend to periodically update and improve recount-methylation with new freezes of the most recent DNAm array experiments from GEO. We expect recount-methylation will help enable research reproducibility in the epigenetics research community.

METHYL-CPG BINDING DOMAIN PROTEIN 2 (*MBD2*) DEPLETION ATTENUATES PRIMARY TUMOR GROWTH AND METASTASIS IN TRANSGENIC MMTV-PYMT MODEL OF BREAST CANCER

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Methyl-CpG-binding domain protein 2 (MBD2), an epigenetic reader of DNA-methylation marks on the genome, has been implicated in the progression of several types of malignancies. To investigate its role in mammary tumor formation and metastasis to distant sites, we generated transgenic mammary tumor virus-polyoma middle T antigen (MMTV-PyMT) mice (C57BL/6 genetic background) having heterozygous (*PyMT;Mbd2*$^{+/-}$) and homozygous (*PyMT;Mbd2*$^{-/-}$) ablation of the allele encoding for full length functional MBD2 protein. MBD2 is upregulated in mammary tissue from MMTV-PyMT mice. The female *PyMT;Mbd2*$^{-/-}$ mice showed a significantly longer survival rate compared to the wildtype (*PyMT;Mbd2*$^{+/+}$) and *PyMT;Mbd*$^{+/-}$ groups. In addition, there was a gene-dose dependent reduction in primary tumor volume and weight when the animals from all three groups (*PyMT;Mbd2*$^{+/+}$, *PyMT;Mbd2*$^{+/-}$, and *PyMT;Mbd2*$^{-/-}$) were sacrificed on week 20. Analysis of lung tissues from different groups revealed a significant decrease in visceral metastasis in the *PyMT;Mbd2*$^{-/-}$ group compared to the control *PyMT; Mbd2*$^{+/+}$ mice. RNA-Sequencing based transcriptome analyses of the primary tumors obtained from *PyMT;Mbd2*$^{+/+}$ and *PyMT;Mbd2*$^{-/-}$ mice showed alterations in the expression of both protein-coding and non-coding genes involved in molecular signaling pathways related to tumorigenesis and metastasis which thereby suggests the direct role of MBD2 in breast cancer progression. Proteomic analysis of the tumor tissues obtained from *PyMT;Mbd2*$^{+/+}$ and *PyMT;Mbd2*$^{-/-}$ showed upregulation of proteins involved in programmed cell death pathways and downregulation of several proteins involved in epithelial to mesenchymal transition. To our knowledge, this is the first study that demonstrates the functional role of a DNA-methylation reader protein in breast cancer using a gene knockout based molecular genetics approach. Results from this study will provide the rationale for further development of first-in-class targeted epigenetic therapies (Epi-therapies) against MBD2 to inhibit the progression of breast and other common cancers.

DIFFERENTIAL EFFECTS OF FOLIC ACID SUPPLEMENTATION IN CORRECTING ASSISTED REPRODUCTION ASSOCIATED ABNORMALITIES IN PLACENTAL DNA METHYLATION AND MRNA EXPRESSION

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The number of children conceived by assisted reproductive technologies (ART) worldwide now exceeds 7 million. Among them, a higher frequency of adverse perinatal outcomes, often associated with epigenetic perturbations, has been reported. Using a mouse model, our group recently showed that ART is associated with increased embryonic developmental delay, a decrease in methylation at certain imprinted loci and global DNA methylation perturbations, the epigenetic defects being more pronounced in female placentas and partially corrected by moderate maternal folic acid supplementation. Here, our goal was to determine if the observed placental DNA methylation defects were accompanied by alterations in gene expression. We used high-throughput RNA sequencing (RNA-seq) to examine the effect of ART in combination with folic acid supplementation on gene expression in female placentas. CF1 female mice were fed diets containing 3 levels of folic acid supplementation (control: 2 mg/kg, moderate: 8 mg/kg or high: 20 mg/kg diet) for 6 weeks prior to natural mating or ART (superovulation, in vitro fertilization, embryo culture and transfer), continuing throughout gestation. Placentas from mid-gestation normal female embryos were collected and 5-6 representative samples chosen for each group. Differential analysis of gene expression performed using RNA-seq revealed that placentas from ART-conceived embryos exhibited 41 significant differentially expressed genes, 17 being up-regulated and 24 down-regulated, when compared to the control group. In contrast, Reduced Representation Bisulfite Sequencing identified a total of 2693 genes with DNA methylation perturbations (2434 being hypomethylated and 259 hypermethylated). Contrary to genome-wide DNA methylation, where folic acid supplementation corrected 8.7% and 7.1% of ART-induced perturbations (low and high dose, respectively), no correction of gene expression was observed with either dose in the placenta. Current analysis is being performed to identify the biological functions involved and to correlate the gene misregulation to the placenta DNA methylome perturbations induced by ART. Taken together, these results suggest that the adverse ART effects we observed are mediated primarily via effects on the DNA methylome rather than secondary to effects on the placenta transcriptome.

$G\beta_1\gamma$ REGULATES ANGIOTENSIN II TYPE I RECEPTOR-MEDIATED TRANSCRIPTION THROUGH THE SWI/SNF CHROMATIN REMODELING COMPLEX

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Cardiac fibroblasts are critical for the formation of a fibrotic scar in damaged areas of the heart following myocardial infarction and in cardiac remodelling during the development of heart failure. Activation of angiotensin II (Ang II) type I receptors (AT1R) initiate signalling pathways involving the Gβγ subunits of heterotrimeric G proteins, leading to induction of a pro-fibrotic gene expression program. Whereas inhibition of canonical Gβγ signalling attenuates the fibrotic response and reduces cardiac remodelling, the role of recently described non-canonical nuclear functions remains unknown. For example, we have identified $G\beta_1\gamma$ at ~800 promoters by chromatin immunoprecipitation (ChIP)-on-chip and a novel interaction with RNA polymerase II (RNAPII) in HEK 293 cells.

The objective of our study was to determine the role of our novel Gβγ interaction with RNAPII in regulation of pro-fibrotic transcription using neonatal rat cardiac fibroblasts. In response to AT1R activation by its endogenous agonist Ang II, we identified an increased interaction between Gβγ and RNAPII by co-immunoprecipitation. To determine the functional impact of this interaction on transcription, we assessed gene expression following $G\beta_1$ knockdown using a fibrosis qPCR array. $G\beta_1$ knockdown led to a potentiated response to Ang II suggesting negative transcriptional regulation by $G\beta_1\gamma$ under normal conditions. ChIP-qPCR demonstrated Ang II-mediated recruitment of $G\beta_1\gamma$ to fibrotic genes identified in the array, further supporting a direct role in transcriptional regulation. Preliminary analysis of a $G\beta_1$ ChIP-seq experiment to assess genome-wide recruitment aligns with our ChIP-qPCR data.

As RNAPII travels along genes, various complexes are recruited to the transcriptional machinery to regulate distinct steps in the transcription cycle. For example, the multi-subunit SWI/SNF chromatin remodelling complex alters chromatin structure to promote RNAPII transcription. We identified the SWI/SNF catalytic subunit SMARCA4 as an interactor for $G\beta_1\gamma$ by affinity-purification followed by mass spectrometry. Co-immunoprecipitation of Flag-$G\beta_1\gamma$ and SMARCA4-GFP overexpressed in HEK 293 cells confirmed the interaction. Furthermore, RNA-seq of our HEK 293 CRISPR $G\beta_1$ KO cell lines predicted SWI/SNF complex activity to be upregulated in the absence of $G\beta_1$, indicating a potential negative regulation of the complex. These two lines of evidence suggested that $G\beta_1\gamma$ may act through this complex to regulate AT1R-mediated gene expression. Preliminary evidence shows that endogenous Gβγ and SMARCA4 interact in cardiac fibroblasts. Taken together, our studies reveal $G\beta_1\gamma$ as a novel regulator of RNAPII transcription, potentially through negative regulation of a SWI/SNF complex.

SIGTOOLS: VISUALIZING GENOMIC SIGNALS

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The availability of sequencing-based genomics assays has led to an explosion in availability of genomics data sets. Deriving insights from these complex data sets require visualization tools. Genome browsers are effective at visualizing a single locus, but analyzing multiple loci requires looking at each locus in turn. There is a need for visualization tools that aggregate information from across the genome and thus allow for the discovery and validation of hypotheses about genomic activity.
We present SigTools, a visualization tool for genomic signals. A genomic signal is a data set that is represented as a vector over the genome. Genomic signals can be derived from a signal data set (such as histone modification signal) or from an integrative continuous annotation approach such as epigenome-SSM.
SigTools can produce 5 types of plots:

1) **Histogram**: Plots the frequency of each value of the signal.
2) **Auto-correlation**: Plots the spatial consistency of a genomic signal by displaying the correlation between a signal and itself at varying genomic distances.
3) **Cross-correlation**: Plots the correlation between each pair in a collection of signals.
4) **Heatmap**: Plots the correlation between each pair in two different collections of signals.
5) **Aggregation**: Plots signal value around a specific annotation point or region.

HARNESSING EPIGENETICS TO IMPROVE MUSCLE STEM CELLS FUNCTIONS IN DUCHENNE DYSTROPHY

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Duchenne Muscular Dystrophy (DMD) is an incurable progressive myopathy caused by the absence of the dystrophin protein (Hoffman *et al*., 1987). This disease is characterized by permanent cycles of muscle damage and regeneration. Previous studies in mouse models suggest an exhaustion of Muscle Stem Cell (MuSCs) (Sacco *et al*., 2010). Our laboratory had obtained clinical muscle biopsies from DMD patients at diagnosis and age-matched normal individuals. Moreover, our laboratory prior results indicate that human primary DMD cells lose their expression of CD56 (used as a myogenicity marker) twice as fast as healthy cells. Gene expression from healthy and DMD muscle cells was quantified using a microarray. The results of this analysis showed that genes involved in the epigenetic modification of chromatin and protein binding of DNA are down-regulated at the time of the loss of myogenicity in normal MuSCs. Strikingly, we observed that many of these genes encoding epigenetic factors are also down-regulated in DMD MuSCs prior to their loss of myogenicity. These results suggest that a down-regulation of genes involved in the epigenetic regulation of cell identity occurs in DMD MuSCs, and that may account for their rapid loss of myogenicity.
Gene expression of epigenetics enzyme during loss of myogenicity in healthy MuSCs was confirm using qPCR. It allows us to confirm underexpression of CBX1, CBX3, H2AFV, SMARCA2 and SMC3. This is correlate with a down expression of these genes in DMD compare to healthy MuSCs. In order to rescue the myogenicity of DMD MuSCs, lentiviral particles were designed to over-express these target genes. After a screen of a series of four promoters to determine which was most active in human MuSCs, EF-1a promoter was selected as the ideal promoter for the over-expression of the cDNAs in primary human MuSCs during several weeks. First results of DMD MuSCs transduction showed a better maintenance of myogenicity in DMD MuSCs after over-expression of 4 different epigenetics factors, SMC3, HP1g, H3F3B or H2AFV, suggesting their role in the maintenance of myogenic cell identity.

UNIQUE EPIGENETIC PROGRAMMING DISTINGUISHES REGENERATIVE SPERMATOGONIAL STEM CELLS IN THE DEVELOPING MOUSE TESTIS

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In the mammalian testis, spermatogonial stem cells (SSCs) sustain steady-state spermatogenesis leading to the production of 85-100 million sperm per day by an adult man. SSCs both self-renew to perpetuate the stem cell pool and give rise to progenitors that enter steady-state spermatogenesis to generate male gametes in the mammalian testis. The extent to which SSCs and progenitors represent distinct spermatogonial subtypes, and whether, in addition to SSCs giving rise to progenitors, progenitors may revert back to SSCs, are unresolved questions. The *Id4-egfp* transgenic mouse model facilitates FACS-based isolation of SSC-enriched/progenitor-depleted and SSC-depleted/progenitor-enriched spermatogonial subpopulations, functionally validated by presence (SSCs) or absence (progenitors) of the capacity to seed spermatogenesis following transplantation to a recipient testis. We examined genome-wide patterns of a) gene expression by RNA-seq, b) six different histone modifications – H3K4me1-3, H3K9me1, H3K27me3 and H3K27ac – by ChIP-seq, c) chromatin accessibility by ATAC-seq, and d) DNA methylation by MeDIP-seq in SSC-enriched ID4-eGFP^{bright} and progenitor-enriched ID4-eGFP^{dim} subpopulations of spermatogonia from the developing mouse testis at 6 days postpartum (P6). While genes expressed at similar levels in both spermatogonial subtypes showed no differences in epigenetic programming, we found consistent differences in epigenetic landscapes associated with genes differentially expressed in the two spermatogonial subtypes. Differential enrichment of H3K27me3 or H3K27ac at promoters, along with differential enrichment of H3K4me1 or H3K27ac at enhancers, as well as differences in distal intergenic low-methylated regions, appear to be the most consistent epigenomic parameters distinguishing

differentially expressed genes. Motif enrichment analysis predicted transcription factors that regulate or mediate this spermatogonial subtype-specific epigenetic programming, and gene-specific ChIP and immunohistochemistry analyses confirmed subtype-specific differences in binding of these factors to target genes and relative abundance of these factors in each subtype, respectively. Collectively, these results suggest that SSCs and progenitors are distinct spermatogonial subtypes differentially programmed to either self-renew and maintain regenerative capacity as SSCs or lose regenerative capacity and commit to spermatogenic differentiation as progenitors.

HISTONE H3 LYSINE 4 MONO-METHYLATION FACILITATES CELL FATE TRANSITIONS DURING MUSCLE REGENERATION

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The histone H3 lysine 4 mono-methyltransferase MLL4 (KMT2D) has been identified as a major histone modifier required for the activation of enhancers. While it has been shown that MLL4 and its corresponding H3K4 monomethylation mark are dispensable for cell identity, the methyltransferase is essential for cellular differentiation in embryonic stem cells. However, the role of MLL4 and the subsequent mono-methylation of H3K4 in the regeneration of adult tissue is not as well established. Here we examine role of H3K4 mono-methylation in muscle regeneration by using an inducible mouse model to specifically ablate MLL4 in satellite cells as well as over expressing a lysine-4-to-methionine mutation (K4M) that inhibits H3K4 methylation. We show that the loss of MLL4 as well as the overexpression of K4M results in impaired regeneration of muscle after acute cardiotoxin induced injury. The loss of MLL4 did not affect the fate of the quiescent satellite cells. However, ablation of MLL4 in satellite cells impaired their ability to activate in response to injury. Similarly, the loss of the H3K4 methylation marks did not affect myoblast proliferation but did impair myotube formation. This suggests that H3K4 mono-methylation is required for efficient transitions between cellular fates. RNA-seq analysis identified ~400 genes that are differentially expressed in MLL4 knockdown cells during differentiation, the majority of which are downregulated. ChIP-seq data shows that MLL4 occupancy during differentiation increased ~10 fold primarily binding at distal intergenic regions. In summary, our results indicate that binding of MLL4 is required to mediate changes in gene expression that mediate differentiation. Taken together, our results show that the mono-methylation of H3K4 is not necessary to maintain cell identity but is required for the cell fate transitions during muscle regeneration.

SYNERGISTIC ANTI-CANCER EFFECT OF A COMBINATION OF S-ADENOSYLMETHIONINE (SAME) AND IMMUNE CHECKPOINT INHIBITORS (ICPI) IN A SYNGENEIC MOUSE MODEL OF MELANOMA.

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Melanoma is one of most diagnosed cancer worldwide and is associated with a high rate of mortality. Recently, treatment with ICPi targeting immune checkpoint ligands PD-L-1,2, (programmed death (PD) ligand 1 and 2) expressed on the surface of tumor cells and their receptor PD-1 on T cell surface has shown marked success in advanced melanoma. In previous studies we have shown that the universal methyl donor SAMe has significant anti-cancer effect in different cancers, however the effects of SAMe on melanoma progression has thus far not been evaluated. Interestingly, SAMe is also known as an immune modulator, essential for T cells activation and proliferation and could potentially cooperate with ICPi and block cancer progression.
In the current study, we examined the anti-tumor effects of SAMe and ICPi inhibitors alone and in combination setting *in vitro* and *in vivo* in a syngeneic mouse model of melanoma. In *in vitro* studies, treatment of mouse melanoma cells B16-F1 with SAMe and anti-PD-L1 antibody showed significant effects on blocking tumor cell proliferation and colony formation. In *in vivo* studies following subcutaneous inoculation of B16-FI cells into female C57BL/6 mice, treatment of animals with either SAMe or anti-PD-1 antibody resulted in significant reduction in tumor volume as compared to control animals. This decrease in tumor volume was significantly higher in animals treated with a combination of SAMe+anti-PD-1 antibody where >50% of animals showed tumor regression. Both SAMe and anti-PD-1 antibody decreased lung metastasis following intravenous inoculation of B16-F1 cells. However, the number of metastatic foci were significantly lower in animals treated with a combination of SAMe+anti-PD-1 antibody. RNA-*seq* analysis of the primary tumors showed changes in the expression of 60 (2 up and 58 down -regulated genes) and 404 genes (262 up and 142 down -regulated genes) following treatment with SAM or anti-PD-1 antibody respectively. However, 1743 genes were differentially regulated (887 up and 847 downregulated genes) following SAMe+anti-PD-1 antibody combination treatment. Consensus PathDB pathway analysis showed downregulation ($p < 0.05$) of key intracellular signaling pathways related to cancer, muscle biology, cell cycle, DNA repair, immune system while upregulated ($p < 0.05$) pathways were related to mRNA processing, translation, metabolism and transcription. Expression of top five up- and down-regulated genes was further validated by RT-qPCR analysis. Tumor immunophenotyping showed significantly higher rate of CD8+T cells proliferation and increased IFN-g cytokine production ($p<0.05$) following SAMe+anti-PD-1 treatment. Further in depth immunophenotyping of primary tumors, lymph nodes and spleens from experimental animals are currently underway.

Our studies showed for the first time that SAMe blocked melanoma growth and metastasis and that a combination treatment with ICPi had a marked effect in blocking melanoma progression and alteration of key genes implicated in cancer and immune response pathways. These finding provide the rationale for the initiation of clinical trials with SAMe and ICPi, both of which are currently approved agents in humans for examining their potentially additive or synergistic effects in patients with melanoma and other common cancers to reduce cancer associated morbidity and mortality.

TARGETING ADAR1 AS THE NEGATIVE REGULATOR OF VIRAL DSRNA SENSING PATHWAY, POTENTIATES THE ANTI-TUMOR EFFECTS OF DNMTI IN COLORECTAL CANCER

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In the past few decades, pharmacological inhibitors of DNA methyltransferases have shown clinical anti-tumor activity against certain hematological malignancies and solid tumors. Their primary mechanism of action has shown to be reactivation of genes, that have been silenced by DNA methylation and re-expression of transposable elements (TEs). We have previously reported that in colorectal cancer cells treatment with low dose of the DNA methyltransferase inhibitor (DNMTi), *5-Aza*-2'-deoxycytidine (5-AZA-CdR), leads to a viral mimicry state and targets cancer initiating cells (CICs) through the accumulation of double-stranded RNA (dsRNA), resulting in activation of RIG-I-MDA5 viral sensing pathway and induction of IFN-stimulated genes (ISGs).
Although low doses of 5-AZA-CdR has anti-tumor effects there is a delay in the clinical response to low doses of DNMTis. In this study, we propose a novel target for activation of viral dsRNA sensing pathway. We found that down-modulation of Adenosine deaminase acting on dsRNA (ADAR1) in CIC enriched patient derived colorectal cancer cells induced the ISGs at higher level, leading to an earlier and more durable response to low dose of 5-AZA-CdR. Furthermore, loss of function of ADAR1 in synergy with DNMTi profoundly reduced CICs *in vitro* and *in vivo*.
Therefore, these findings suggest ADAR1 inhibition in combination with DNMTi as a novel therapeutic strategy to potentiate the antiviral response in colorectal cancer cells.

INDEX AND BIOLOGICAL SPECTRUM OF ACCESSIBLE DNA ELEMENTS IN THE HUMAN GENOME

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DNase I hypersensitive sites (DHSs) are generic markers of regulatory DNA and harbor disease- and phenotypic trait-associated genetic variation. We established high-precision maps of DNase I hypersensitive sites from 733 human biosamples encompassing 439 cell and tissue types and states, and integrated these to precisely delineate and numerically index ~3.6 million DHSs encoded within the human genome, providing a common coordinate system for regulatory DNA. Here we show that the expansive scale of cell and tissue states sampled exposes an unprecedented degree of stereotyped actuation of large sets of elements, signaling the operation of distinct genome-scale regulatory programs. We show further that the complex actuation patterns of individual elements can be captured comprehensively by a simple regulatory vocabulary reflecting their dominant cellular manifestation. This vocabulary, in turn, enables comprehensive and quantitative regulatory annotation of both protein-coding genes and the vast array of well-defined but poorly-characterized non-coding RNA genes. Finally, we show that the combination of high-precision DHSs and regulatory vocabularies markedly concentrate disease- and trait-associated non-coding genetic signals both along the genome and across cellular compartments. Taken together, our results provide a common and extensible coordinate system and vocabulary for human regulatory DNA, and a new global perspective on the architecture of human gene regulation.

SUPPORTING EQUITY DIVERSITY AND INCLUSION IN THE LIFE SCIENCES

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In academic Life Sciences diversity and inclusion remains an issue for women, LGBTQ and non-binary individuals, and underrepresented or marginalized groups from different racial, ethnic and indigenous backgrounds. In Biology, PhD graduates are fairly well balanced between women and men yet, at the Assistant Professor level women make up only about 20-30% of Biology Professors. A study published last year analyzed the representation of women at early and later career stages in different science and medicine sub-disciplines and suggested that it could take longer than 50 years for equal representation of women without intervention. This trend of underrepresentation at the professorial level persists even in fields where women are equally represented at the PhD and postdoctoral career stages. Representation from other groups is much lower and again

unlikely to change without close attention to the underlying causes and structural barriers in place that limit Equity Diversity and Inclusion (EDI) in the Life Sciences. At the same time research shows that diverse teams are more productive indicating that we are missing talented individuals and important perspectives that would drive innovation due to this lack of representation. One of the factors that limits progression to the highest career stages for these individuals are biases in publishing, recruitment and invitations to speak at international meetings. A recent article published by Nature describes some of the ways in which these biases can remain, even for organizations with a strong commitment to EDI. As funding bodies and universities are increasingly asking applicants to address EDI requirements knowledge of the issues and potential solutions is important to our community. I will present data on the current state of EDI in the Life Sciences and solutions that have been effective to increase inclusion and remove barriers.

ROLE OF SETDB1/H3K9ME3, AND ITS READER HP1 PROTEINS IN DNA METHYLATION AND SILENCING OF GERMLINE GENOME-DEFENSE GENES

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Germ cell precursors are solely responsible for ensuring successive generations by giving rise to spermatozoa and oocytes. In mouse embryos, primordial germ cells (PGCs), which are the progenitor cells for spermatogenesis and oogenesis, are specified from undifferentiated epiblast cells at around embryonic day (E)6.5, and thereafter start to migrate to gonads. In migrating PGCs, specific expression of the germline genome-defense genes (essential for transposon suppression in the germline either via piRNA biogenesis or post-transcriptional regulation) including *Dazl*, *Ddx4* and *Piwil2* are initiated at E10.5. During embryogenesis, *de novo* DNAme occurs globally until E6.5, but genomic DNA is almost completely demethylated in gonadal PGCs, reaching a low point at E13.5. DNAme is reported to repress germline defense genes in the epiblast and early PGCs, and thereafter, concomitant with global loss of DNAme, these genes are demethylated in late PGC development. Notably, we found that in embryonic stem (ES) cells, germline defense genes were not only highly DNA-methylated, but also bound by SETDB1, and enriched for H3K9me3 and HP1 proteins. However, kinetics between H3K9me3 and DNAme during *in vivo* embryogenesis/PGC development are poorly understood. Furthermore, the role of the "H3K9me3 reader" HP1 proteins in silencing of germline defense genes has not been studied.
To determine the relationship between H3K9me3 and DNAme *in vivo*, we first analyzed the data from ultra-low-input (ULI)-ChIPseq and whole genome bisulfite sequencing (WGBS), and found that H3K9me3 was deposited prior to *de novo* DNAme during embryogenesis. We therefore have hypothesized that SETDB1/H3K9me3/HP1s play a role in silencing of germline defense genes prior to deposition of *de novo* DNAme and are required for establishment/maintenance of DNAme in the epiblast/early PGC development.
To verify that H3K9me3 is deposited prior to DNAme during embryogenesis/PGC development, we derived PGC-like cells (PGCLCs) from ES cells via epiblast-like cells (EpiLCs) and found that the EpiLC/PGCLC culture system recapitulates the kinetics of H3K9me3 and DNAme establishment *in vivo*. We also showed that the germline defense genes are derepressed upon *Setdb1*-knockout (KO) or *HP1α/β/γ*-KO during EpiLC/PGCLC induction, suggesting that SETDB1/H3K9me3 and HP1s are required for silencing of these genes.
We are currently carrying out bisulfite analyses in *Setdb1*-KO and *HP1α/β/γ*-KO PGCLCs to determine whether SETDB1/H3K9me3 and/or HP1s are required for establishment/maintenance of DNAme in the epiblast/ during early PGC development.

IMPUTATION-AIDED GENERATION OF IHEC REFERENCE-QUALITY METHYLOMES

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Genome-wide DNA methylation (methylome) analysis is of broad interest to medical research because of its central role in human development and disease. However, generating reference-quality methylomes on a large scale is particularly expensive due to technical issues inherent to bisulfite sequencing, requiring deeper than usual sequencing. To address this limitation, we have explored *in silico* imputation methodologies to improve the coverage and quality of whole-genome bisulfite sequencing (WGBS) data and demonstrate its use for the generation of reference-quality methylomes, meeting the standards of International Human Epigenome Consortium (IHEC).
Imputation is a statistical technique where missing values are substituted with computed values. The process requires reference data from which the missing information can be extracted and imputed to boost data quality and facilitate integrative analysis using metadata. Recently published imputation tools for WGBS include DeepCpG (Angermueller et al. 2017) and BoostMe (Zou et al. 2018). DeepCpG utilises deep learning techniques to determine associations between DNA sequence patterns (1kb) and between neighbouring CpG sites (50 CpG sites). BoostMe uses a gradient boosting algorithm to extract useful information from the two immediately, neighbouring CpG sites.
We assessed the performance of DeepCpG and BoostMe as well as generic machine-learning techniques using high-quality (~100x) WGBS data from IHEC. High to low coverage datasets were simulated by down-sampling this dataset on the read-pair level and missing values were imputed. As the actual methylation value is known for each missing data-point in the down-sampled dataset, the accuracy of the imputed value can be calculated. Our results show that with a simulated dataset of ~10x, both DeepCpG and BoostMe are able to impute the methylation values of an additional ~12.5 million CpG sites with acceptable accuracy (DeepCpG: 6.11% MAE; 13.48% RMSE; BoostMe: 6.26% MAE; 11.69% RMSE).
As methylation of neighbouring CpGs is highly correlated in a genomic distance-dependent manner (Eckhardt et al. 2006), we also developed a much simpler and faster approach termed Global Imputation of Mean cpg MEthylation (GIMMEcpg). By calculating the distance-weighted mean of the two immediately

neighbouring CpGs, GIMMEcpg outperformed DeepCpG and BoostMe in accuracy and speed, by imputing 13.1 million CpG sites (5.76% MAE and 11.46% RMSE). With additional distance filtering (1kb), fewer CpG sites (8.8M CpG sites) can be imputed with an even higher accuracy (5.49% MAE; 11.1% RMSE).
To demonstrate its potential, we used GIMMEcpg to impute ten shallow (17x) whole blood WGBS datasets, from healthy volunteers of the Personal Genome Project UK, to the equivalent of IHEC reference methylomes of greater than 60x. These ten methylomes (complemented by 30x WGS and RNA-seq) could constitute the first IHEC methylomes, from a clinically relevant human tissue, available under open access.

ASSIGNING BIOLOGICAL SEX TO INDIVIDUAL HUMAN BRAIN CELLS BASED ON SINGLE NUCLEUS CHROMATIN ACCESSIBILITY PROFILES.

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Background: The brain is the most complex organ with numerous different cell types, including many that are yet to be clearly defined. Since their initial development, single-cell sequencing technologies have proven to have immense potential with broad applications for brain research. Unfortunately, because of the small amount of material provided by each cell, the amplification steps required for adequate sequencing can bias results. This, in addition to the high price of commercial droplet-based technologies, represents a significant barrier to the generation and interpretation of single-cell sequencing results. One way to circumvent both of these issues would be to multiplex samples. Some researchers have attempted to use SNPs as a means of multiplexing however, the sparse sequencing coverage, the 3' bias in transcriptomic studies and multiplets have made it difficult to tease apart samples afterward. Ensuring that individual sample genomes are different enough to tease them apart post sequencing is the simplest way to combine samples to reduce noise and cost.
Aim: The aim of this study is to use the chromatin accessibility profiles of the sex chromosomes to identify the biological sex of each individual cell mixed on a single lane of sequencing from a droplet-based capture experiment.
Methods: We isolated nuclei from post-mortem brain tissue, producing a nuclei suspension suitable for transposition. Nuclei from a male and female brain were counted and mixed at equal proportions and at a skewed ratio of 2.3 female: 1 male to test the sensitivity of the identifying sex in-silico. Combining different sexes in a single capture could technically be applied to any tissue type.
Results: We were able to accurately identify and separate male and female nuclei at the expected proportions based only on chromatin accessibilities patterns. We were also able to identify noise associated with the lane specific amplification of the samples, improving the quality and accuracy of the information.
Conclusions: Mixing samples of different sexes is a feasible method to account for variability introduced by lane specific capture and for reducing capture costs.

ASCL1 FACILITATES THE NEUROENDOCRINE LINEAGE REPROGRAMING THROUGH REMODELING OF CHROMATIN LANDSCAPE

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Background: Continued androgen receptor (AR) signaling after Castration Resistant Prostate Cancer (CRPC) had ushered the need for AR inhibitors. These highly effective AR pathway inhibitors (ARPIs) such as Enzalutamide (ENZ) have enjoyed great success as they increase patient survival. However, they play a role in emergence of a more aggressive, non AR-dependent phenotype known as treatment induced neuroendocrine prostate cancer (tNEPC). With the exception of alterations in *RB1* and *TP53,* which are known to render cells more amenable to lineage reprogramming, few other genetic differences are observed between adenocarcinoma (Adeno) and NEPC. Suggesting, an epigenetic dysregulation may underlies this conversion. We identified that ASCL1 is slightly upregulated at the basal level following RB1 knockdown and its expression accelerates with ENZ. Integrating RNA-seq data, we confirmed the upregulation of ASCL1 expression and transcriptional activity, in NEPC cell lines and patient tumors. Our preliminary data shows that overexpression of ASCL1 alone is sufficient to induce a NEPC phenotype in CRPC cell model as measured by increased expression of NE markers; this effect was further enhanced in the context of RB1 loss. Conversely, silencing ASCL1 reduced the expression of NE markers across NEPC cell models as well as reduced cell growth. Recent studies identified EZH2 as a key player in regulating the transition to NEPC through epigenetic modifications by targeting the H3k27me3. Interestingly, integrating our ChIP-seq data discovered that NEPC specific EZH2 binding sites were often bordered by ASCL1 motif, suggesting an interplay between EZH2 and ASCL1. Our preliminary data shows overexpression of ASCL1 in Adeno cells increases H3k27me3, while knockout of ASCL1 resulted in downregulation of H3k27me3. The relationship between ASCL1 and EZH2, if any, is still unknown. However, our data suggest that they might cooperate to reprogram the chromatin landscape in order to activate the neurogenesis transcriptional program.
Hypothesis: ASCL1 facilitates a change in the chromatin landscape to support a transcriptional program driving neuroendocrine prostate cancer.
Method: Measuring the chromatin accessibility by H3k27me3 and H3k27ac ChIP-seq and chromatin nucleosome positioning (ATACseq) during ENZ induced transition of Adeno to NEPC and overlay it with ASCL1 and EZH2 ChIP-seq.
Conclusion: We have discovered that downstream of RB1 loss, several molecular events such as differential activation of EZH2 and upregulation of ASCL1 play a major role in the neuroendocrine differentiation by changing the chromatin

landscape. We aim to identifying the interplay between EZH2 and ASCL1 in mediating chromatin architecture changes and the mechanism behind it. Data from our laboratory and others suggest that ASCL1 may drive early transcriptional and epigenetic reprogramming induced by ARPIs opening up potential therapeutic avenues for combining targeting ASCL1 with ARPIs in CRPC to prevent emergent NEPC.

TISSUE-SPECIFIC CHROMATIN ACCESSIBILITY SIGNATURE AT THE SINGLE-CELL LEVEL RESOLUTION

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Epigenetic mechanisms such as post-translational histone modifications can determine whether DNA would be accessible to regulatory elements during transcription. Mapping accessible chromatin that is shown to have significant impact on response to DNA damage, hypoxia and various treatment modalities is crucial for the definition of epigenomic makeup in every tissue type and can facilitate prediction of cell behavior in different biological conditions.
In this study, we aimed to characterize accessible chromatin regions using the Assay for Transposase-Accessible Chromatin (ATAC-seq) to identify tissue-specific chromatin accessible regions in various organs and assign them to unique cell clusters representing each tissue type.
Characterization of chromatin accessibility was performed using single-cell ATAC-seq in 75,823 cells from 13 specimens including 8 different tissue types in mouse and human. Tested tissue specimens include brain, lung, colon, pancreas, heart, kidney and liver. Samples were processed using 10x Genomics protocol for scATAC-seq. Generated data was analyzed using Cell Ranger pipeline. To assess whether single cell data recapitulates DNA accessibility in bulk tissue, aggregated single cell data were compared against bulk ATAC data from the same specimen. Unique peaks representing each tissue type were identified and were linked to their corresponding cell cluster.
Chromatin accessibility profiling in a panel of multiple tissue types at the single-cell level can identify shared accessible regions which helps to better understand commonalities in chromatin architecture. Further, tissue-specific open chromatin regions could as well shed light on the therapeutic strategies toward a more organ-specific targeting of the disease or cancer site leading to less off-target result on uninvolved tissues.

DEVELOPING HYBRID METHODS TO CHARACTERIZE NUCLEOSOME STRUCTURE AND DYNAMICS WITH HIGH PRECISION

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Nucleosomes are basic units of chromatin compaction and hubs in epigenetic signaling pathways. Nucleosomes experience a broad repertoire of alterations that affect their dynamics and interactions with various binding partners. To gain insights into intrinsic dynamics of nucleosomes we apply all-atom microsecond molecular dynamics simulations on microsecond timescale and develop hybrid approaches by combining experimental data with molecular modeling and molecular dynamics simulations.
Our first approach, HYDROID, allows the interpretation of DNA-protein interactions by quantifying Hydroxyl Radical Footprinting data and by integrating it with atomistic structural models. We applied HYDROID to characterize yeast centromeric nucleosome of unknown structure and identified the precise positioning of centromeric DNA sequence. In another study we used HYDROID to pinpoint the footprint of interaction between the inner kinetochore protein Mif2/CENP-C and centromeric DNA which helps to identify specific binding mode to AT-rich DNA.
Our second hybrid approach was applied to describe histone octamer distortion upon processive movement of the ATPase motor of chromatin remodeler ISW2 on nucleosomal DNA. We performed accelerated molecular dynamics simulations guided by experimental chemical crosslinking data to gain an understanding of the potential perturbations occurring in the nucleosome structure and investigate how nucleosomal DNA can adjust to perturbations in the histone octamer structure.

EXPLORING INTERACTIONS OF NUCLEOSOME VIA INTERACTOME ANALYSIS AND INTEGRATIVE MODELING

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Interactions between nucleosome and various chromatin factors play key role in chromatin regulation such as histone modification and chromatin remodeling. Taking advantage of advances in a number of experimental techniques such as cro-EM, NMR and crosslinking mass spectrometry, we constructed three comprehensive human nucleosome interactomes using high-resolution experimental structures from PDB, recent crosslinking mass spectrometry data in human nuclei and experimental data form APID database. We explored the interactions of nucleosomes with binding partners via network analysis at different levels of details including residue-level, domain-level and protein-level. Residue level interactome allowed us to identify well-defined binding hotpots on histone and nucleosomal DNA. In addition, we performed extensive all atom molecular dynamic simulations to explore the histone tail dynamics and conformations in the context of nucleosomes with linker DNA. Histone tails were found to interact with nucleosomal DNA and histones via preferred binding modes affecting DNA solvent accessibility and thus potentially competing with other nucleosome binding partners.

KDM1A MEDIATES TRANSGENERATIONAL METABOLIC DISTURBANCES IN A SEX-SPECIFIC MANNER AND IS LINKED TO DIET-INDUCED ALTERATION IN SPERM CHROMATIN SIGNATURES

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Obesity occurs in 650 million people worldwide. Known factors contributing to obesity risks include genetics, lifestyle and maternal factors. Epidemiological studies and animal model data indicate that paternal diet affects offspring risks for metabolic disorders. However, the mechanisms underlying this non-genetic inheritance of complex metabolic disease remain elusive. Alterations in histone methylation in sperm have been implicated in transgenerational epigenetic inheritance and offspring health (Siklenka et al., 2015). This was previously demonstrated using a transgenic mouse model that overexpresses the histone demethylase enzyme KDM1A specifically in the germline, giving rise to males with an abnormal sperm epigenome. Using this genetic model combined with a diet-induced obesity model, our Objectives were to: 1) investigate whether epigenome-environment interactions can lead to enhanced metabolic phenotypes transgenerationally, and 2) whether transmission of phenotypes can be linked to sperm histone H3 chromatin signatures. Experimental Approach: KDM1A transgenic males (F0) with a pre-existing compromised sperm epigenome and C57BL6NCrl wildtype sires, were exposed to either a low- or high-fat diet (10% or 60% kcal fat, respectively) for 10-12 weeks. Their regular chow-fed descendants (F1 and F2) were tested for metabolic phenotypes and the liver for differential gene expression. Sperm of sires fed a HFD or controls was subjected to chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) targeting a modification on histone H3* and analyzed to identify diet-modulated changes in enrichment which could be implicated in offspring phenotypes. Results: F0 males fed a high-fat diet became obese, glucose intolerant and insulin insensitive irrespective of their genotype. Intergenerational effects of paternal diet were observed in male offspring only, while metabolic functions in female descendants were not impacted by paternal diet. Males sired by obese transgenics had enhanced metabolic phenotypes compared to wildtype obese descendants. Interestingly, transgenerational effects of high-fat diet were only observed in transgenic descendants, suggesting that paternal exposure to a multitude of environmental stressors may exacerbate descendant risks to develop obesity and metabolic syndrome. Sperm chromatin profiling revealed diet-induced alterations in enrichment. Differentially enriched regions occurred at genes with functions corresponding to observed offspring phenotypes, including genes involved in placenta development, as well as glucose and lipid metabolism. Liver transcriptomic data analysis are ongoing. This is the first report linking high-fat diets and alterations in the sperm epigenome at the level of a histone modification. These findings shed light on the potential contribution of chromatin in sperm in paternal transmission of complex diseases.
*patent pending

INVESTIGATION OF DNA ELEMENTS THAT ENABLE ESCAPE FROM X-CHROMOSOME INACTIVATION

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X-chromosome inactivation (XCI) is an epigenetic process that transcriptionally silences all but one X chromosome in mammalian cells. Established in early development, the inactive X (Xi) remains stably silenced through further mitotic divisions. Notably, 15-25% of human and 3-7% of mouse X-linked genes are still expressed from the Xi, effectively escaping XCI. Bioinformatics studies have correlated certain genetic elements and epigenetic marks with escape from XCI, but there is currently a paucity of functional validation experiments, and no model is fully predictive of escape status. A recent study in our lab has demonstrated that our mouse model can properly recapitulate the inactivation status of an *Hprt*-integrated bacterial artificial chromosome (BAC) containing the human escape gene *RPS4X*. It is particularly notable that the mouse *Rps4x* gene is subject to inactivation, suggesting there are elements governing escape present in the human DNA construct and absent from the endogenous mouse *Rps4x* locus. We are currently investigating two candidate regions that contain DNA elements potentially involved in the escape of *RPS4X*. Targeted CRISPR/Cas9 has been employed to delete one of these regions in both male and female human somatic cells. The male somatic cells were used to determine if this candidate region is vital to ongoing *RPS4X* expression from the active X chromosome, in which case this region would not be specifically informative about escape from the Xi, and a new candidate region would be selected. In this case, the candidate region did not extinguish expression of *RPS4X* in the male somatic cells, and so investigation continued. Similarly, the female somatic cells were used to investigate the maintenance of *RPS4X* expression from the Xi. Escape status was monitored with promoter DNA methylation, reverse transcriptase quantitative PCR (RT-qPCR), and RNA fluorescence *in situ* hybridization (RNA-FISH). It is also possible that the regulatory effects of this candidate region occur during early development, when XCI is established. For this reason, we will next delete this candidate region in our mouse embryonic stem cells (mESCs) containing the transgenic BAC. These mESCs can then be differentiated to initiate XCI and determine the effect of this region on the establishment of escape for *RPS4X.* Definitively identifying regions that enable *RPS4X* to escape from XCI will advance investigation into the mechanistic processes that establish an area of active transcription on the epigenetically silenced Xi, furthering knowledge about transcriptional regulation as a whole.

CHARACTERIZING SS18-SSX FUSION ONCOPROTEIN DEPENDENCY IN SYNOVIAL SARCOMA AND ITS ENHANCER LANDSCAPE

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Background: Synovial sarcoma is an aggressive soft-tissue malignancy that predominately affects adolescents and young adults. This cancer is characterized by a pathognomonic balanced chromosomal translocation t(X;18)(p11.2;q11.2) which results in the production of the fusion oncoprotein, SS18-SSX. Native SS18 is a component of the mammalian SWI/SNF (mSWI/SNF) or BAF complex, a chromatin remodeller. Previous research has shown that the mSWI/SNF complex plays an important role in regulating enhancers, which are enriched in H3K27ac when active. We hypothesize that the fusion protein exploits SWI/SNF to dysregulate enhancers but is not needed to maintain the enhancer landscape. Ultimately, a better understanding of SS18-SSX mediated oncogenic mechanisms will reveal therapeutic vulnerabilities.
Methods: Using nucleosome density chromatin immunoprecipitation sequencing (ndChIP-seq) for the 6 core IHEC histone modifications, including H3K27ac, we have profiled 7 cases of primary synovial sarcoma. We complemented these data with whole genome bisulfite sequencing and RNA-seq to profile the methylome and transcriptome of these tumors, respectively. Additionally, we have similarly profiled 7 synovial sarcoma tumors from 2 independent mouse models expressing human SS18-SSX. Publicly available ChIP-seq and RNA-seq data were obtained for control and SSX knockdowns (KD) in human synovial sarcoma cell lines including Aska, HSSY2 and SYO-1.
Results: Genome-wide H3K27ac density segregated primary synovial sarcoma cases from various other normal and diseased tissues profiled by IHEC. We identified a set of genomic regions enriched in H3K27ac that are shared and specific to synovial sarcoma. Surprisingly, active enhancers showed a greater proportion of shared sites between samples (~50%), compared to super-enhancers (~25%; a subset of highly active enhancers) in both primary human tissue and the mouse synovial sarcoma model.
H3K27ac signal at shared enhancers did not decrease after KD of SSX in the Aska cell line. Correspondingly, the expression of genes associated with these enhancers and upregulated in synovial sarcoma compared to other soft-tissue sarcomas (TCGA) and was not decreased after KD of SSX in synovial sarcoma cell lines.
A set of shared transcription factor binding motifs, such as those belonging to the ETS family, were identified in H3K27ac-enriched regions in human and mouse synovial sarcoma. Across cases, motifs found in distal enhancers were more variable than those found in promoters and may correspond to subtypes of the disease.
Conclusion: Synovial sarcoma genomes possess a shared set of specific enhancers compared to a compendium of normal and diseased tissues. Neither the shared enhancer set, nor expression of associated genes is altered after KD of the fusion oncoprotein in synovial sarcoma cell lines. This lack of continued dependence on the oncofusion for maintenance of pathogenic enhancers has implications for therapeutic strategies which aim to target the oncofusion directly. Additionally, differences in distal enhancers may correspond to subtypes of synovial sarcoma.

ZYGOTIC DE NOVO DNA METHYLATION OF THE PATERNAL GENOME

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With the exception of CpG islands (CGIs), the sperm genome is densely methylated in mammals. Following fertilization, the paternal genome undergoes rapid DNA methylation (DNAme) loss before the first S-phase, while the maternal genome shows relatively delayed replication-coupled demethylation. Paradoxically, further supporting the distinct epigenetic trajectories of the parental genomes, recent mass spectrometry evidence revealed that a low level of de novo DNAme may also be occurring in mouse zygotes exclusively on the paternal genome. However, the genomic loci involved and consequences for transcriptional regulation at such loci was not addressed.
Employing allele-specific analysis of whole-genome bisulphite sequencing data, we show that several dozen CGI promoters are de novo methylated on the paternal genome in 2-cell stage embryos, a subset of which maintain such DNAme in the blastocyst. Furthermore, a number of loci showing such zygotic paternal DNAme acquisition (PDA) are hypermethylated in androgenetic blastocysts but hypomethylated in parthenogenetic blastocysts, corroborating PDA. Strikingly, PDA is lost following maternal depletion of DNMT3A, and ectopic transcription of many hypomethylated PDA genes was observed from the cognate paternal allele at the 4C stage. Such aberrant transcription was lost by the ICM stage, coincident with demethylation of the associated promoter. Taken together, these observations uncover a novel role of maternal DNMT3A activity in post-fertilization epigenetic reprogramming of the paternal genome.

STRAND-SPECIFIC NON-CPG METHYLATION PATTERNS ACROSS GENIC REGIONS

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Non-CpG methylation (mCH) of DNA is a cell type-specific epigenetic mark with roles in cellular differentiation and disease (Jang et al. 2017). Unlike mCG, mCH cannot be maintained across

both strands by DNA methyltransferase 1 (DNMT1), so mCH can be attributed to the *de novo* DNMTs, DNMT3A/B. The distribution and patterns of mCH can help elucidate its biological role, and help characterize DNMT3A/B. To this end, we averaged the methylation signal for different trinucleotide contexts (mCGN, mCHH, etc.) across genomic regions of interest, focusing on contexts associated with DNMT3A (mCAH) and DNTM3B (mCAG) in mouse embryonic stem cells.
Upon separating the methylation signal by strand, we found that mCH was higher along the negative (transcribed) strand than the positive (non-transcribed) strand across entire genes. Strand-specific peaks and valleys also appear across intron-exon junctions. Interestingly, when stratifying the genes by expression level, we find the difference in methylation between strands is positively correlated with gene expression. No such differences were observed for mCG.
The histone transferase NSD1 is the main enzyme responsible for dimethylation of H3K36 in intergenic regions, while the K36me2 mark recruits DNTM3A. Using data from NSD1 KO cells, where DNMT3A is relocated entirely to genic regions, we observe overall increased non-CpG methylation within genes, and we also see that the asymmetry of *de novo* methylation is exaggerated compared to wild type cells.
The association of strand-specific mCH with rates of transcription raises two hypotheses on causality which we intend to investigate: i) Does transcription machinery occupancy of the transcribed strand affect strand-specific mCH and *de novo* DNMT recruitment? or ii) Do *de novo* DNMTs and mCH have a function in transcription? The mCH patterns at intron-exon junctions leads to similar questions about mCH and splicing. We also intend to control for potential nucleotide biases that could lead to the observed strand asymmetry of *de novo* methylation, either in terms of the methylation contexts themselves or the sequences which flank methylation contexts.

KDM4A INHIBITION INDUCES A SENESCENCE-LIKE PHENOTYPE AND INCREASES SENSITIVITY TO BCL2 INHIBITORS IN MLL-AF9 ACUTE MYELOID LEUKEMIA

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Epigenetic modifications regulate gene expression, genome stability, and nuclear architecture. A better understanding of the implications of epigenetic differences in oncogenic cells is a promising strategy in the development of new therapeutics and clinical care. We have determined that KDM4A, an epigenetic regulator of the JUMONJI lysine demethylase family, is overexpressed in tissues from MLL-AF9 pediatric acute myeloid leukemia (AML) patients. We aim to characterize the precise epigenetic function of KDM4A in pediatric MLL-AF9 AML and its role in modulating gene expression and chromatin architecture. We are also investigating the potential of molecular inhibition of KDM4A as a novel therapeutic strategy in AML treatment. The loss of KDM4A in MLL-AF9 AML cell lines resulted in decreased cell proliferation, increased expression of markers of differentiation, and repressed transcription of genes involved in leukemic maintenance, including leukemic-required c-Myc. We also observed an increase in chromatin repressive histone modifications at the Myc super-enhancer, a lineage-specific genomic element required for the robust expression of the Myc oncogene, as well as a decrease in the recruitment of BRD4, an epigenetic remodeling factor required for super-enhancer activity. Our results also indicate that KDM4A-depleted or inhibited cells express markers of senescence, including increased levels of anti-apoptotic Bcl-2 family proteins. Notably, the inhibition of KDM4 proteins followed by treatment with Bcl-2 inhibiting compounds enhanced apoptosis in MLL-AF9 leukemic cells. In AML, malignant leukemic stem cells have an unlimited potential for self-renewal and are blocked in an undifferentiated state. Our results demonstrate that KDM4A inhibition and depletion leads to growth arrest and a senescence-like phenotype in MLL-AF9 leukemic cells in vitro. Our study will decipher a precise molecular role of KDM4A in the maintenance of leukemic proliferation and describe a potential novel therapeutic approach for treatment of MLL-AF9 AML, a disease still plagued with dismal survival rates.

ACCURATE AND COST-EFFECTIVE DISCOVERY OF ACTIVE GENE REGULATORY INTERACTIONS USING ARIMA-HICHIP

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Precise spatiotemporal control of gene regulation is fundamental to normal biological processes and the development of disease. Therefore, detection of gene regulatory interactions is a critical component of understanding the biology of gene regulatory elements. Proximity ligation assays coupled with NGS provide a means to measure chromatin interactions within the nucleus. For example, Hi-C measures the frequency of genomic interactions at a genome-wide scale and has become a mainstream technology for detecting genome structures ranging from genomic compartments, to TADs, to chromatin loops, collectively undercovering a structural framework for gene regulation. Despite this utility, HiC is a genome-wide sequencing assay with relatively increased costs for high resolution analyses (>600M reads). Furthermore, HiC doesn't directly enrich for gene regulatory interactions, such as those between promoters and enhancers. To address this and build upon our existing Arima-HiC kit platform, we have developed Arima-HiChIP. Arima-HiChIP integrates our Arima-HiC technology with ChIP-seq, thereby enriching for gene regulatory interactions associated with active regulatory elements. The optimized Arima-HiChIP workflow begins with ~2 million cells and can be completed in 3-4 days. Our Arima-HiChIP workflow is currently designed for integration with both Covaris and Diagenode chromatin shearing instruments. We have optimized the Arima-HiChIP workflow for

high resolution analysis of gene regulatory interactions associated with histone modifications, such as H3K27ac or H3K4me3, with reduced sequencing requirements (<200M reads). We have also built-in QC assays for the HiC portion of the workflow, chromatin shearing efficiency, ChIP enrichment, and library complexity. On the bioinformatics side, we have validated two open-source HiChIP analysis pipelines, MAPS and FitHiChIP. From extensive analyses of public and internal HiChIP datasets, we propose a new standardized framework and metrics for analytical QC of HiChIP data. We aim to expand our Arima-HiChIP technology towards optimized protocols for analysis of transcription factors and low input samples.

EFFICIENTLY QUANTIFYING DNA METHYLATION FOR BULK- AND SINGLE-CELL BISULFITE DATA

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Background. Whole Genome Bisulfite Sequencing (WGBS) is considered the gold standard for genome wide, high resolution DNA methylation measurements. With the ongoing advances in Next Generation Sequencing techniques, for example single-cell methylomes, large amounts of sequencing data are produced for different tissues, and cell types. However, while sequencing throughput has increased, alignment and methylation calling algorithms have not been adapted to the increasing demands, which causes a serious bottleneck. Furthermore, widely used tools do not resolve the mapping ambiguity introduced by WGBS, which comes at the cost of accuracy of the called methylation rates.
Results. Here, we present a novel approach called FAME (Fast and Accurate MEthylation calling), which combines bisulfite read alignment and methylation calling into one task. We designed a specialized index structure based on sequence k-mers that can store mammalian sized genomes efficiently, while allowing fast lookups of candidate matching positions for bisulfite converted reads. We further designed fast filters to prune redundant or repetitive information in the index, which drastically reduces the search space for read alignment.
Using this index, we can find candidate matching regions for a read by looking up all gapped k-mers of a queried read efficiently using rolling hash functions. Smart filtering of the candidate regions based on q-grams allows us to reduce time spent on false positive candidates. To carry out exact indel-based alignment, we extended the Shift-And based pattern matching automata to allow for asymmetric C/T mapping to resolve ambiguity introduced by bisulfite conversion. Once a unique best alignment is found, methylation levels are directly estimated in the data structure, thus avoiding excessive I/O for writing large Bam files as well as additional postprocessing time for methylation calling.
We compared FAME against the state of the art with synthetic and real data sets. The synthetic data consisted of 25 million reads sampled from the human chromosome 22 mimicking the WGBS protocol, such that ground truth methylation rates were known. The real data included 437 million PE WGBS reads and EPIC beadchip microarray data for LNCaP cells taken from Pidsley et. al (Gen Biol 2008), where the EPIC array measurements were taken as ground truth. Difference to ground truth values was computed using root mean square error (RMSE) over all existing CpGs. The quality of the methylation rates of FAME are on par with the most accurate competitor, while FAME provides an order of magnitude faster processing time. FAME also supports single cell data and running FAME on a dataset of 190 cells took ~13 hours compared to >5 days for all other methods tested.
Conclusion. We suggested a new method, FAME, for calling methylation rates of WGBS data that is based on a novel index structure specifically tailored for methylation data. We proved on both synthetic and real data that the quality of methylation rates called by FAME are on par with the most accurate state of the art aligner, but FAME processes data an order of magnitude faster. Furthermore, due to the novel index structure, FAME does not require extensive I/O or realignment and thus naturally suits the task of aligning extensive WGBS single cell data. Hence, FAME paves the way for methylation calling of large-scale datasets and is ideal for cloud computing. FAME is open source and free to use and can be downloaded from https://github.com/FischerJo/FAME.

HNF1A-AS1: A PUTATIVE ONCOGENE

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Background: Hepatocyte nuclear factor 1 homeobox A antisense RNA 1 (HNF1A-AS1), a long non-coding RNA, is expressed in numerous types of cancers and is associated with poor clinical outcomes such as higher mortality rates, greater metastatic capacity, and poor prognosis of the disease. It is also involved in metabolic disorders such as metabolic syndrome and fatty liver disease. HNF1A-AS1 is mostly localized to the nucleus and based on this subcellular localization it is thought to interfere with gene expression. In this study, we examined the expression level and localization of HNF1A-AS1 RNA, HNF1A RNA, and HNF1A protein has been investigated in both cancer cells and in pancreatic beta cells (the insulin-secretion cells of the Islets of Langerhans).
Methods: A total of four human colon cancer cell lines, including HT29, HTC116, RKO, and SW480, and a normal colon cell line CCD841 along with a lung cancer cell line A549 were cultured. Additionally, we used MIN6 cells, a clonal cell line of pancreatic beta cells. Western blot was done on all samples to quantify HNF1A protein level. RT-qPCR was done to illustrate expression level of HNF1A-AS1 and HNF1A genes in total cell lysates as well as subcellular compartments.
Results: Subcellular localization of HNF1A-AS1 varied in different cell types. In adenocarcinomic human alveolar basal epithelial cells (A549) it is localized in cytoplasm while in colorectal adenocarcinoma cells (HT29) is mostly localized in the nucleus. HNF1A protein is mostly located in nuclear compartment, while HNF1A RNA is commonly cytoplasmic.
Conclusion: HNF1A-AS1 is located in different compartments in various cells which might affect cellular function in different ways.

REGIONAL DNA METHYLATION PATTERN ANALYSIS IN EPIGENOME-WIDE ASSOCIATION STUDY

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DNA methylation is an important epigenetic biomarker for investigating human complex disease susceptibility. The identification of potential biomarkers is typically explored through epigenome-wide association studies (EWAS). However, current EWAS mainly focus on individual CpGs by comparing the average level among a group of cells, few studies have conducted to investigate the DNA methylation pattern variations with regarding to cell-to-cell heterogeneity, especially in a haplotype manner. With the cost-effective methyl-Capture sequencing (MCC-Seq) technology available recently, it become feasible to investigate the DNA methylation haplotypes in EWAS. Here, we proposed a computational pipeline to process haplotype-based DNA methylation data association analysis. We designed local clusters of CpG sites (LCCSs) to pre-define genome-wide methylation haplotype block regions (MHBs) and applied entropy – measuring the cell-to-cell heterogeneity, methylation haplotype level (MHL) as well as average methylation frequency (AMF) on these MHBs. We demonstrated the pipeline on a recently published sperm cohort. We identified 118,326 MHBs with average length of 235bp across 48 samples. We performed the EWAS based on entropy, MHL and AMF profiles for comparing the effect of a common polymorphism (677CC vs TT) in methylenetetrahydrofolate reductase (MTHFR) as well as folic acid supplementation (6 months) in infertile men. Revisiting the DMRs identified through traditional individual CpGs based EWAS, the median of the entropy for these DMRs ranged from 0.38 to 0.45 while the background cell-to-cell variance in this cohort is 0.17, indicating cell-to-cell variations may confound the EWAS results. Compared with single CpG based EWAS analysis results, MHL based EWAS signals show less cell-to-cell variances (e.g. entropy of 0.19 vs. 0.45 in folic acid supplementation effect comparisons). Moreover, majority of the MHL-based DMRs were complementary to the individual-CpG based DMRs. The gene ontology enrichment analysis revealed that MHL-based DMRs were enriched in alpha-Linolenic acid metabolism pathway (p-value<10^{-5}) and vascular smooth muscle contraction (p-value <10^{-4}) etc. in MTHFR TT patients with or without folic acid supplementation, while for MTHFR CC patients, they are enriched in Hippo signaling pathway (p-value <10^{-8}), long-term depression and MAPK signaling pathway (p-value<10^{-5}) etc. Taken together, our results implied not all sperm cells contribute to the environmental influences in a same way, cell-to-cell variations need to be carefully considered. Our analysis pipeline could also easily apply to single-cell methylation data for precise identification of cell-specific methylation biomarkers.

IWATE TOHOKU MEDICAL MEGABANK EPI-GENOME COHORT: CONSTRUCTION OF DNA METHYLATION REFERENCE PANELS OF BLOOD CELLS AMONG JAPANESE POPULATIONS

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The Great East Japan Earthquake (GEJE) that occurred on March 11, 2011, caused severe damage to Pacific coastal areas of Japan. The Iwate Tohoku Medical Megabank Organization (IMM) was established with the aim of reconstructing the medical system in disaster-struck areas, supporting disaster victims, and realizing the potential of personalized medicine using genomic and epigenomic information. The IMM recruited 32,913 participants in Iwate prefecture between May 2013 and March 2015 (baseline survey). Questionnaire forms, physiological function test results, and biological specimens were collected from all participants. A secondary survey was initiated in 2017 (four to five years after the baseline survey) to assess change over this time period in the health status of participants and the multiomics data they provided.
For the baseline survey, we collected six types of purified blood cells (monocytes, B cells, CD4-positive T cells, CD8-positive cells, NK cells, and neutrophils) from 143 individuals, peripheral blood mononuclear cells (PBMCs) from 4,025 subjects, and whole blood samples from 32,871 participants. In the secondary survey, we collected 2,295 PBMC samples, and 13,422 whole blood samples prior to April 2019, and scheduled the collection of up to 4,000 PBMCs and 24,000 whole blood samples by the end of March, 2021.
We conducted multi-omics analysis of 102 CD4-positive T cell samples, 102 monocyte samples, and 94 neutrophil samples by whole-genome sequencing, whole-genome bisulfite sequencing, and whole transcriptome sequencing. Based on DNA methylation (DNAm) profiles, we estimated average DNAm levels and variation covering ~24 million autosomal CpG sites. We constructed an integrative database, and released the data on our open website (iMETHYL: http://imethyl.iwate-megabank.org/statistics.html) which provides information on inter-individual DNAm variation calculated by two methods (standard deviation (SD), and reference interval (RI)—defined as the difference between the 95th and 5th percentiles of the DNAm level among individuals). Furthermore, we performed DNAm analysis of 384 PBMCs by capture-based, targeted bisulfite sequencing, and whole transcriptome sequencing. Using these multi-omics data, we conducted genome-omics association analyses [expression quantitative trait locus (cis-eQTL), expression quantitative trait methylation (cis-eQTM), and methylation quantitative locus (cis-mQTL) analyses] for each of three types of cells, and for PBMCs. The summarized association data has been published on our iMETHYL website.
Our iMETHYL website provides a control dataset for epigenome association studies and also provides a tool for annotating susceptibility SNVs identified by genome-wide association

studies. We will obtain further multi-omics data from the secondary survey to make our database more comprehensive.

DISTINCT HISTONE MODIFIERS SHAPE THE METHYLOME IN THE MATERNAL VS PATERNAL GERMLINE

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DNA methylation (DNAme) is implicated in the etiology of developmental disorders as well as in cancers and is required for male germline development. While this epigenetic mark is highly dynamic during embryonic and germ cell development, it is propagated stably to daughter cells upon cell division to maintain transcriptional integrity. Following global erasure of DNAme in primordial germ cells, *de novo* DNAme patterns are established in a sex-dependent manner. DNAme in oocytes occurs within transcribed regions enriched for H3K36me3 while that in sperm occurs broadly in H3K4me3 depleted regions. However, the molecular mechanisms shaping sexually dimorphic DNAme patterns in the germline have been poorly understood. Using low-input ChIP-seq and whole-genome bisulfite sequencing (WGBS) methods, we recently showed that in mouse oocytes the H3K36 KMTase SETD2 is required for H3K36me3 and *de novo* DNAme by DNMT3A/3L, indicating that H3K36me3 acts upstream of DNAme in the female germline (Xu et al., 2019). Surprisingly however, we found that *de novo* DNAme in the male germline is independent of SETD2. Using ChIP-seq data generated from developing male germ cells and intersecting those with published WGBS data, we found the regions that gain DNAme in the male germline are marked by H3K36me2, with only a subset also marked by H3K36me3. Furthermore, deletion of *Nsd1*, another H3K36 KMTase, in male germ cells results in widespread DNA hypomethylation at H3K36me2 depleted regions, indicating H3K36me2 directs male germline DNAme. In summary, together with our previous work (Xu et al., 2019), this study reveals sexually dimorphic difference in histone H3K36 methylation states shapes the distinct patterns of germline DNAme.

INTEGRATING HI-C DATA WITH FUNCTIONAL GENOMICS ASSAYS FOR CONTINUOUS GENOME ANNOTATION

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The 3D architecture of DNA in the nucleus plays a central role in many cellular processes, including gene regulation and DNA replication. 3D architecture and related processes can be queried using sequencing-based assays including Hi-C and ChIP-seq.
Segmentation and genome annotation (SAGA) methods are widely used to jointly model diverse genomic data sets. These algorithms take a collection of genomics data sets as input and output an annotation of the regulatory state of every position in the genome. However, most existing SAGA algorithms cannot incorporate chromatin conformation information (although some modifications have been proposed that alter the underlying probabilistic model). Such information can be measured by the Hi-C assay, but it is represented as a 2D matrix over the genome, rather than a 1D signal track.
We aim to incorporate Hi-C into SAGA methods by first reducing the Hi-C matrix to a small number of tracks. This is possible due to a recently developed method called SNIPER. SNIPER is a method that uses a denoising autoencoder followed by a classifier to compartmentalize a genome. A nutoencoder is a type of neural network that can represent information of input data in a latent variable with much smaller size.
We aim to convert 2D Hi-C data to a small number of 1D tracks using SNIPER, combine it with other 1D assays like ChIP-Seq and DNase-Seq, then use a SAGA method to produce a genome annotation. This annotation will more result in more accurate and informative classification for genome compartmentalization and labeling.

INVESTIGATING THE AGE-RELATED CHANGES IN GENOME ORGANIZATION USING HUTCHINSON-GILFORD PROGERIA SYNDROME IPSCS.

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Lamins form the primary component of the nuclear lamina (NL); a structure indispensable for chromatin organization and genome maintenance. Specifically, lamins anchor heterochromatin to the NL to form compartments termed lamina-associated domains (LAD), although Lamin A is involved in genome wide chromatin assembly of hetero- and euchromatin. In Hutchinson-Gilford Progeria Syndrome (HGPS), a mutation (G608G) within Lamin A leads to the expression of an abnormally spliced protein termed Progerin. HGPS patients exhibit aging phenotypes early after birth and perish in teenagerhood due to cardiovascular complications. Hallmark cellular features of HGPS include abnormal nuclear structure, loss of peripheral heterochromatin and redistribution of epigenetic marks; features associated with aged individuals. However, the molecular mechanisms underlying the observed genomic alterations in HGPS have yet to be explored globally. We previously generated an induced pluripotent stem cell (iPSC) model of HGPS which recapitulated the accelerated aged phenotypes observed in HGPS pathology and demonstrated an increased occurrence of molecular features associated with aging (oxidative stress, DNA damage). Using this model, we assessed the age-related genomic changes by conducting genome-wide mapping of chromatin organization (Hi-C) in HGPS vascular smooth muscle cells at early and late passages. Analysis of the interaction maps (50 Kb) suggests differences in compartment state (active vs inactive) in HGPS. Further annotation analyses suggest alterations in chromatin organization (DNA compartments and loops) in HGPS. With higher Hi-C sequencing

depth and the incorporation of multiple datasets (ChIP-seq, ATAC-seq), we expect to uncover the epigenetic drift driving the observed genomic and transcriptomic changes.

MCGILL EPIGENOMICS MAPPING CENTRE: A CANADIAN PARTNERSHIP

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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 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 to the scientific community 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, histone modification using chromatin immunoprecipitation (ChIP-Seq and ChIPmentation) and assay for transposase accessible chromatin (ATAC-Seq). When sample processing is completed, the platform provides to the collaborators, in addition to the raw data, a complete quality control (QC) report which includes sequencing stats/metrics and UCSC browser tracks for quickly assessing the quality of the data.
A new mandate for the mapping centre renewals (2017-2022) 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 generated significant interest and a competitive peer-review of the applications identified selected projects for further analysis. Among the four projects that were selected for reference epigenome mapping at the McGill EMC platform, one has been completed and two are ongoing.
Aside from the community samples program, McGill EMC works closely with collaborators on various projects by providing technical and analytical expertise. To address the specific collaborator's needs, modifications to the core EMC pipelines were introduced to accommodate either low input material (e.g. frozen tissue sections), challenging cell types (e.g. adipocytes), large tissue samples, and high throughput needs. Recent McGill EMC collaborators including Drs. Tomi Pastinen (Children's Mercy Hospital, Kansas), Luis Barreiro (U. Chicago), Elin Grundberg (CMH, Kansas), David Labbé (McGill U.), Sven Bailey (McGill U.) and Paz Polak (Mount Sinai, New York) demonstrate the role of the McGill EMC platform in serving the broad epigenetic community.

TRANSCRIPTION FACTOR BINDING SEQUENCE DIVERSITY IS A REQUIRED FEATURE OF MAMMALIAN ENHANCERS WHICH CAN BE USED FOR SEQUENCE-BASED ENHANCER PREDICTION IN MULTIPLE CELL TYPES

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Non-coding regulatory regions are critical for normal development and often inappropriately regulated in disease contexts, yet we have only a limited understanding of these regions at a sequence level. To better understand the transcriptional regulatory code, we identified embryonic stem (ES) cell enhancers using mouse-human comparative epigenomics and machine learning. Regions with conserved binding of multiple transcription factors (TFs) in mouse and human were found to be enriched in the number of unique transcription factor binding sequences (TFBS) conserved across five mammals. Enriched TFBS were both expected based on the ChIP-seq data used and novel regulators of the pluripotent state. The accuracy of these predictions was confirmed using enhancer reporter assays and site directed mutagenesis of conserved TFBS in natural enhancers as well as the construction of synthetic enhancers. Previous approaches to evaluate the activity of synthetic enhancers in ES cells revealed an optimal combination of 4 TFBS; however, testing this sequence compared to a natural enhancer revealed limited activity for the 4 TFBS sequence. As we found that natural enhancers have on average 12 unique conserved TFBS we hypothesized that >10 unique TFBS are important for regulatory activity. Multiple synthetic enhancers constructed from >10 unique TFBS had robust enhancer activity whereas sequences containing <10 unique TFBS or 14 repetitions of one TFBS had no activity compared to a minimal promoter alone. These findings reveal a required feature of mammalian enhancers, TFBS diversity above a threshold. Based on this requirement for unique TFBS in mammalian regulatory regions we developed a sequence-based enhancer prediction model which can be applied to any cell-type for the identification of non-coding regulatory regions and novel TF regulators. This model predicted enhancers for heart, liver and brain tissues in humans and identified regions with links to genetic diseases and cancer.

PRMT7: A NEW TARGET FOR CANCER IMMUNOTHERAPY

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Exploration in the field of epigenetics has revealed roles for protein arginine methyltransferases (PRMTs) as major players in normal development and disease thus positioning these enzymes as a new class of therapeutic targets. Arginine methylation is a post-translational protein modification catalyzed by a family of nine protein arginine methylmembers (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, a class III of PRMTs is the focus of my study, and it has been implicated in histone methylation and regulation of gene expression. Recent evidence reveals the relatively high expression patterns of PRMT7 in many cancer types and this elevated expression in cancer correlates with metastasis and oncogenesis (Baldwin et al., 2015).

Human tumors have developed multiple strategies to escape the host immune system. Evasion of the immune system is a hallmark of cancer and one common mechanism by which this occurs is a critical immune checkpoint regulated by programmed death ligand-1 (PD-L1), expressed on cancer cells and its receptor, programmed death-1 (PD-1), expressed on T-cells. However, how and when PD-1/PD-L1 pathway is regulated in melanoma remains less understood. To date, no study has examined the role of arginine methylation by PRMT7 in the sensitivity of skin tumors to immunotherapy, thus elucidating the exact relationship between PRMT7 and immune checkpoint regulation may aid in developing a clinically approach aimed at reducing PRMT7 in different cancers to promote cell death

Here, we show for the first time that loss of PRMT7 increases the sensitivity of tumor cells to immune attack by boosting the immune response to fight cancer. We validate the proof-of-concept in vitro, by showing a significant decrease of the IRF1 protein in melanoma cells that are deficient in PRMT7, which in turn decreases PD-L1 mRNA and protein level. Furthermore, in a mouse transplantable tumor model in vivo, we show that mice injected with PRMT7-null B16-melanoma develop smaller or no tumors and survive longer than control mice. Moreover, by Flow Cytometry, we show that melanomas devoid of PRMT7 are more infiltrated with CD8+ T cells, and less infiltrated with myeloid derived suppressor cells and therefore we would now like to assess the potential clinical significance of our findings by using patient-derived-melanoma samples in order to establish reliable correlations between PRMT7, immune and clinical responses, facilitating the development of skin cancer biomarkers.

Because of the crucial roles that arginine methylation plays in cancer, selective inhibitors are in high demand, not only for enzyme activity inhibition but also for therapeutic avenues to boost anti-tumor-immunity. Based on my project, I believe that combining PRMT7 inhibitors with anti-PD-L1/PD-1 therapy might be a promising approach to overcome the resistance to tumor immunity and to help more patients.

CHARACTERIZATION OF ENHANCERS AND OPEN CHROMATIN REGIONS IN SMALL CELL CARCINOMA OF THE OVARY HYPERCALCAEMIC TYPE (SCCOHT)

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Background: Mutations to subunits of mBAF, a chromatin remodelling complex, can be a sole recurrent genetic feature in rare cancer subtypes. Small cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) is one such example characterized by a loss of the catalytic subunit of the BAF complex, SMARCA4. Based on the previously reported role of BAF in the establishment and maintenance of active enhancer states we hypothesize that the loss of SMARCA4 in SCCOHT leads to a pathogenic enhancer state which drives tumour initiation.

Methods: Four primary SCCOHT tumour specimens, confirmed to be both SMARCA4 and SMARCA2 negative via immunohistochemistry were subjected to reference epigenomic profiling following IHEC standards. The human ovarian granulosa tumour cell line, COV434 was engineered to express SMARCA4 (COV434-S4) in response to doxycycline treatment. RNA-seq and ChIP-seq (H3K27me3, H3K27ac, H3K4me3, and H3K4me1) were performed following DOX treatment of COV434-S4 and a matched nonDox-treated in triplicate. These data were complemented with previously published RNA-seq and ChIP-seq datasets generated for the SCCOHT cell-line BIN-67.

Results: While SMARCA4 loss was attributed to mutations, SMARCA2 loss was due to H3K27me3 mediated promoter silencing in both COV434 and in the context of primary SCCOHT tumours. Surprisingly, SMARCA4 re-induction resulted in few H3K27me3 alterations globally (<10 regions). In contrast large alterations in H3K27ac were observed with a with a strong directional increase in total number of marked regions (5197 gained) those with increased signal (7421) in both genetic and intergenic site. A strong directional increase in expression was also observers with 1074 upregulated genes and 3 downregulated genes (FC>2 and p-val <0.05) following SMARCA4 induction. Increased enhancer states were supported by increased H3K27ac and/or increased H3K4me1 in pre-existing Active Enhancers (AEs: 3297) and *de novo* H3K27ac in pre-existing primed (H3K4me1 marked) regions (Primed to Active/PtAs: 4693). In addition, 16,938 open chromatin regions (OCRs) showed increased signal following re-induction, of which 2747 were in PtA and 1468 in AEs. Regardless of direct SMARCA4 association as determined by ChIP-seq, OCRs with PtA or AEs (AeOCR) correlated with higher gene expression. Motif analysis revealed OCRs, PtAs, and AEs were enriched for TEAD binding elements, a subset that contained SMARCA4 binding were also enriched with AP1. Associating AeOCRs to the nearest gene transcription

start site, 85 genes were identified upregulated in COV434-S4 relative to tumours and COV434. Gene enrichment of said genes indicated roles in epithelial differentiation and tissue morphogenesis.
Conclusion: Re-induction of SMARCA4 in SCCOHT cell line model resulted in global induction of H3K27ac, H3K4me1 and OCRs and a reprogramming of the enhancer landscape and transcriptome.

PIONEER TRANSCRIPTION FACTORS FROM DNA METHYLATION PROFILES

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DNA methylation is widely analyzed as a fundamental epigenetic modification to regulate mammalian gene expression, where each type of cell creates specific methylation pattern during its differentiation. While enzymatic mechanisms of DNA methylation (DNMT3A/3B, DNMT1 and UHRF1) and demethylation (TETs and TDG in active demethylation) are well characterized, little is known about how DNA methylation dynamics are spatiotemporally regulated during cellular differentiation. Recently, a subgroup of transcription factors (TFs) termed pioneer TFs have been shown to carry out DNA demethylation in a binding site-specific manner, although small number of pioneer TFs, including RUNX1 by us, have been identified yet. We have developed an informatics pipeline to predict pioneer TFs from DNA methylation profiles derived from whole genome bisulfite sequencing (WGBS). The pipeline consists of four steps, preparation of bins, extraction of differentially methylated bins, the TF binding motif enrichment analysis, and filtering by TF gene expression. We applied the pipeline to the International Human Epigenome Consortium (IHEC) WGBS data. In comparison with DNA methylation profiles in ES cells, average number of demethylated bins are 100,000 while methylated bins 20,000 in examined cells/tissues, indicating that DNA demethylation is major event in cellular development. We have predicted more than 100 pioneer TFs, in which distinct sets of pioneer TFs are predicted in cell lineage-specific manner. Validation of the predicted pioneer TFs is going on using our developed method that detect DNA demethylation activity of TFs.

ACCOUNTING FOR CELL TYPE COMPOSITION IN BULK TRANSCRIPTOME AND METHYLOME DATA BY USING SCRNA-SEQ REFERENCE

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Gene expression and CpG methylation in patients have been measured in bulk samples of blood, liver, fat, brain etc. Such bulk tissues are mixtures of multiple cell types. Disease association of a gene (or CpG site) might vary by cell type, in which case the average is observed at tissue level. Here, I account for cell type composition in transcriptome and methylome data and refine the associations by cell types.
Cell types and their gene expression profile were obtained by published single-cell RNA sequencing experiments of human. By using gene expression profile of cell types as reference, the cell type composition of each tissue sample was inferred. By including the inferred cell type composition in multiple regression analysis, association of the omics measurements with disease were refined. Published data on asthma, Alzheimer's disease and liver disease were reanalyzed.

DYSREGULATION OF HUMAN ENDOGENOUS RETROVIRUSES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic Lupus Erythematosus (SLE) is a multi-systemic autoimmune disease. SLE patients show a wide range of symptoms and are often misdiagnosed. Currently, the underlying cause of this disease is unclear. Intriguingly, dysregulation of Human Endogenous Retroviruses (HERVs), which are ancestral viral transcripts integrated into the human genome, is strongly correlated with SLE. Bidirectional transcription of ERVs that form into double-stranded RNA (dsRNA) was reported to trigger immune responses through viral mimicry. HERVs Envelope (Env) or Group-specific antigen (gag) protein also have the capacity to serve as neo-antigens and causes molecular mimicry. It was reported that CD4+ T lymphocytes of SLE patients show significantly lowered DNA methylation levels at HERV-E family long terminal repeats (LTRs), concomitant with increased of HERV-E gag transcripts. This epigenetic alteration may be involved in the development of autoimmune response. However, the precise mechanisms of how HERVs can trigger SLE phenotypes remain elusive.
My research examines the epigenetic profile of Peripheral Blood Mononuclear Cell (PBMC) in SLE patients and whether HERVs are differentially expressed. I hypothesized that epigenetic and transcriptional dysregulation of HERVs in SLE patient T cells may contribute to the development of autoimmune responses. Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) and transcriptome sequencing (RNA-Seq) were performed to delineate the genome-wide chromatin states and transcriptomic changes in PBMC, CD4+ and CD8+ T cells in SLE patients. Transposable Elements (TEs) are found to be differentially expressed in SLE patient, associated with increased expression of immune-response genes and altered nearby gene expression. These findings may improve our understanding of how HERVs contribute to SLE pathogenesis and potentially drive autoimmune responses. It could also shed lights on the molecular mechanisms of other autoimmune diseases.

DNA HYPER METHYLATION AS A FUNCTION OF HISTONE MODIFICATION: DOES SINGLE RADIATION INDUCE PERSISTENT EPIGENETIC CHANGES INDEPENDENT TO A GENES' MODIFICATION PROFILE?

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Nowadays stem cell research demonstrates stem cell heterogeneity in many tissues with subpopulation being particular resistant to genomic stress. As an example, stress-sensitive intestinal stem cell (ISCs) have been shown to become replaced by more resistant populations following moderate irradiation. Here, we asked for specific epigenetic profiles of such subpopulations. Performing ChIP-seq, we observed histone modification changes in the intestine of mice weeks after irradiation and/or following Msh2 loss compared to controls. Among the common changes, we identified H3K4me3 recruitment to the promoter of H3K27me3 target genes. Notably, our measured profiles of controls are consistent with data from the mouse ENCODE consortium and data on isolated mouse ISCs. Performing RNA-seq, we find that H3K4me3 recruitment surprisingly is not accompanied by changes of gene transcription. We applied a mathematical model of epigenetic regulation of transcription and show that: i) H3K4me3 recruitment at constant transcription can be explained by increased DNA binding of H3K4me3 and H3K27me3 histone methyl-transferases due to lower promoter DNA methylation and: ii) the recruitment is capable of protecting the genes' promoters against hyper-methylation.
Our results suggest that stress-resistant ISCs are characterized by higher H3K4me3 at H3K27me3 target genes compared to sensitive ISCs. Consequently, they are better protected against promoter hyper-methylation.

SIRTUIN-MEDIATED HISTONE DEACETYLATION MITIGATES RIF1-DEPENDENT REPLICATIVE STRESS IN CELLS WITH SHORT TELOMERES

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Chromatin structure influences the ability of cells to respond to impediments of DNA replication fork progression, i.e., to replicative stress. In the yeast *Saccharomyces cerevisiae*, newly synthesized histone H3 deposited behind DNA replication forks are acetylated on lysine 56 (H3K56ac). Two members of the class III sirtuin family of deacetylases, Hst3 and Hst4, subsequently globally deacetylate this residue after S phase. Cells lacking Hst3 and Hst4 present constitutive H3K56ac, which causes extreme sensitivity to replicative stress via mechanisms that remain poorly understood.
We performed a genome-wide screen to identify haploinsufficent essential genes that promote cell fitness upon nicotinamide (NAM)-induced inhibition of sirtuins. Heterozygosity of genes promoting DNA replication origin licensing and activation, as well as ones preventing telomere shortening, caused NAM sensitivity in a H3K56ac-dependent manner. Interestingly, deletion of the gene encoding Rap1-interacting factor 1 (Rif1), which elevates replication origin activity, suppressed NAM-induced S phase progression defects, along with the temperature sensitivity of *hst3Δ hst4Δ* mutants. Moreover, the strong NAM sensitivity of cells with short telomeres, including pre-senescent telomerase mutants, was suppressed by *rif1Δ*. Since Rif1 elongates telomeres in a telomerase-dependent manner, these results suggest that Rif1 may cause replicative stress in cells with short telomeres by repressing DNA replication origins genome-wide. Consistent with this notion, NAM-treated *est1Δ* and *est2Δ* mutants failed to complete S phase in a Rif1-dependent manner. Together with recent reports indicating that interaction with Rap1 sequesters Rif1 at telomeres, our data support a model in which telomere shortening, by limiting Rap1 binding to telomeric DNA, may globally redistribute Rif1, thereby reducing origin activity genome-wide, compromising S phase progression.

EFFECTIVE PROTEIN BIOCONTAINMENT THROUGH PHASE SEPARATION

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Liquid-liquid phase separation generates specialized compartments throughout the cell, but unlike membrane-bound organelles, their protein contents rapidly exchange. We describe a novel liquid-like state for the lysine methyltransferase KMT5C where it diffuses within heterochromatin condensates but undergoes strikingly limited nucleoplasmic exchange, revealing a barrier to exit similar to that imposed by biological membranes. This retentive behavior was essential to reducing the surface area of these assemblies and mapped entirely to a short protein segment containing multiple determinants for heterochromatin localization. Importantly, KMT5C retention was rapidly reversible and responsive to underlying chromatin state where even modest perturbation led to aberrant spreading of its catalytic product throughout the nucleus and decreased cell viability. From a functional standpoint, this physical partitioning therefore serves as a biocontainment mechanism that exerts strict spatial control over trimethylation of histone H4-lysine 20 by KMT5C. Collectively, our work provides a foundational example of a protein whose activity is sequestered through near complete retention in a liquid condensate and the domain that imparts this property. Moreover, we establish that this unique behavior can be achieved with a remarkably limited number of sequence features and without progression to a gel or solid.

DISTINCT DNA METHYLATION TARGETS BY AGING AND CHRONIC INFLAMMATION

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The origins of epigenetic alterations have a large impact on public health. Aberrant DNA methylation is induced by aging and chronic inflammation, and it is widely believed that inflammation accelerates age-related methylation. Here, we took advantage of the potent methylation induction in human gastric mucosa by *Helicobacter pylori*-triggered inflammation and compared methylation targets by aging and inflammation. Of 270,249 genomic blocks produced from the 482,421 CpG probes on an Infinium 450K microarray, 17% genomic blocks were highly methylated by inflammation, even after correcting for the influence of leucocyte infiltration. 62 % of the hypermethylation was acceleration of age-related methylation, but 22 % was specific to inflammation. Regions with H3K27me3 were frequently methylated both by aging and inflammation. Basal methylation levels were essential for age-related hypermethylation, while even regions with little basal methylation were methylated by inflammation. When limited to promoter CpG islands, being a microRNA gene and having high basal methylation levels strongly enhanced methylation, while H3K27me3 strongly enhanced inflammation-induced methylation. Inflammation-induced methylation was able to override active transcription. In young gastric mucosae, genes with high expression and frequent mutations in gastric cancers were more frequently methylated than in old ones. In conclusion, inflammation-induced methylation was not a simple acceleration of age-related methylation, and unique factors were involved in target specification.

dCYPHER®: A HIGH-THROUGHPUT METHOD FOR INTERROGATION OF CHROMATIN INTERACTING PROTEINS

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Many epigenetic regulators recognize the PTM state of chromatin through evolutionarily conserved histone-binding domains and thereby recruit nuclear complexes to specific loci within the genome. While significant advances have been made in our understanding of how these domains interact with histones, a wide number of putative binding motifs have yet to be characterized, and undoubtedly, novel domains remain to be discovered. Techniques such as histone peptide arrays are widely utilized for the discovery and characterization of histone readers, however, this approach requires significant quantities of material, often lacks robustness, and is not compatible with biologically relevant nucleosomes. To overcome these limitations, EpiCypher® has developed dCypher™, a high-throughput discovery platform for the rapid screening of chromatin interacting proteins (effector proteins, enzymes, and antibodies). This platform is a no-wash bead-based proximity assay (*i.e.* AlphaScreen®) and utilizes comprehensive libraries of singly and combinatorially-modified histone peptides (> 288) and recombinant designer nucleosomes (> 80) encompassing ~100 unique modifications on the four core histones and several histone variants. We have employed dCypher to characterize the binding of many reader domains (i.e. bromodomains, YEATS, PHD, and PWWP domains) producing expanded hit profiles compared to peptide arrays while consuming significantly less material. Further, dCypher has demonstrated its amenability to rapid optimization of chromatin reader binding conditions (i.e. modifying salt concentration or addition of competitor DNA). Intriguingly, through the incorporation nucleosomes we have observed modified binding profiles (enhanced and refined) compared to using peptides. The major advantages of dCypher over conventional methods like histone peptide arrays include: **(1)** much lower starting material (>100-fold); **(2)** higher sensitivity and specificity; **(3)** rapid optimization of binding conditions and, **(4)** compatibility with nucleosomes, and **(5)** considerably less time consuming (> 80% reduction), thus providing a simple yet powerful high-throughput platform for chromatin biology research and drug discovery.

DNA METHYLATION AND LONG-RANGE CHROMATIN CONTACTS AT THE *IRXA* GENE CLUSTER IN HUMAN COCAINE DEPENDENCE.

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Background: Multiple, interacting biological factors are likely to contribute to the development and maintenance of cocaine use disorders. Epigenetic mechanisms are thought to mediate the relationship between chronic cocaine dependence and molecular changes in addiction-related neurocircuitry but have been understudied in human brain. 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 humans.
Methods: We used reduced representation bisulfite sequencing (RRBS) to identify genome-wide differential methylation, in postmortem caudate nucleus tissue from 25 individuals with cocaine dependence and 25 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), HEK293 cells, 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* transcript, and cells that endogenously express *IRX2* and its neighbor *IRX1*, have low levels of *IRX2* methylation. Moreover, we identified a large chromatin loop in human cells, which is related to *IRX2* methylation 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*, and in decreased frequency of long-range chromatin contacts.

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.

ANNOTATING THE HUMAN GENOME BY INTEGRATING OVER A THOUSAND EPIGENOMIC DATASETS

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Epigenomic marks, such as histone modifications, histone variants, open chromatin regions, is a powerful source of information to reveal the locations and dynamics of DNA regulatory regions. In such context, ChromHMM was created to discover the chromatin combinatorial and spatial patterns – denoted chromatin states--, and annotate genomic functional domains, hence assisting studies of various applications. Previously, ChromHMM has been primarily applied in cell-type-specific manners; creating separate functional annotations for different cell types. Currently, given the volume of epigenetic data in an ever-increasing number of cell types, we are interested in investigating the benefits a non-cell-type-specific ChromHMM state genome annotation from training epigenetic signal data in various cell types. In this project, we trained a ChromHMM model using data of 1032 experiments of chromatin marks' signal profiled in 127 reference epigenomes from Roadmap Epigenetics project, to annotate the genome into 100 distinct cross-cell-type chromatin states. The resulting model is termed full-stack model. We conducted various analysis to evaluate the benefits of such a universal annotation in complementing existing annotations to understand numerous regulatory contexts. We showed that the increased complexity of 100-state genome annotation – afforded through augmenting data from numerous cell types – is more predictive of various external annotations such as TSS, CpG Islands, gene body, as compared to using cell-type-specific annotations. The full-stack model's annotation reveals the genomic contexts of variants prioritized by different variant prioritization scores such as CADD, FIRE, and Eigen. Full-stack ChromHMM states provide a useful resource for analyzing the epigenetic landscape of genetic variants.

INTER-SPECIES AND INTER-TISSUE COMPARISONS HIGHLIGHT THE CONTRIBUTION OF A DNA TRANSPOSON 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 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. These NF-kB bound transposons possess multiple active epigenetic marks and reside near TNFα-induced genes. By querying NF-kB binding across multiple tissues, we were also able to identify multiple TE subfamilies that enriched in different tissues. Combining NF-kB binding data across species and tissues, we constantly observed the high enrichment signal from an ancient mammalian DNA transposon, MER81. Closer investigation revealed that the ancestral MER81 sequence has two intact NF-kB binding motifs that are responsible for NF-kB binding. We next confirmed the enhancer activity of MER81 sequence by luciferase assay and surprisingly found the two motifs appear to work synergistically to determine the function. Lastly, we traced MER81 sequence change during ~100 million years of human evolution. We found a constant copy number but stabilized motif occurrences of MER81 during evolution, indicative of a novel adaptation model for ancient transposons. Taken together, our results demonstrated the potential involvement of multiple transposons in establishing NF-kB mediated regulatory networks and highlighted the functional contribution of a conserved DNA transposon during mammalian evolution.

RESTORATION OF BAF COMPLEX MEMBERS REGULATE ENHANCER LANDSCAPE IN MALIGNANT RHABDOID TUMOR AND OVARIAN CLEAR CELL CARCINOMA

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Background: The mSWI/SNF (BAF) complex is a chromatin remodeler often perturbed in oncogenesis and genetic inactivation of one or more of its subunits is observed in a broad array of cancers. Bialliec loss of SMARCB1, a BAF complex member, is pathognomonic in the highly aggressive pediatric cancer malignant rhabdoid tumour (MRT). ARID1A mutation is observed in 50% of the ovarian clear cell carcinoma (OCCC). To elucidate mechanisms by which BAF complex member loss drive transformation in disease we have generated reference epigenome datasets and ATAC-seq in inducible isogenic cell line models.
Methods: To investigate the role of *SMARCB1* and *ARID1A* in the MRT and OCCC, we performed bulk and single cell ATAC-seq, native ChIP-seq (H3K4me3, H3K4me1, H3K27ac, H3K27me3, H3K36me3, H3K36me2, H3K9me3) for histone modification, RNA-seq and whole genome bisulfite sequencing on MRT and OCCC isogenic cell line models: the MRT cell line G401-pInd20 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 105440 and 58095 open chromatin regions (OCRs) in the presence and absence of SMARCB1 respectively, of which 71179 (67.5%) were only found in the G401-B cells. The numbers of active enhancers, as defined by the presence of the H3K27ac and H3K4me1, were also increased from 46239 to 89118 regions following SMARCB1 induction.
We identified open chromatin regions derived from rare single cell populations that are not identified in the bulk, i.e. 29600 and 13183 regions for G401-B1 and G401 cells respectively. High correspondence was observed between promoter/gene body accessibility and expression for genes such as F3, MMP7, and CD44.
AP-1 and TEAD families were specifically enriched within G401-B1 unique OCRs, whereas CTCF and BORIS were specifically enriched in G401 unique OCRs. AP-1, TEAD family and FOXO1 binding motifs were significantly enriched in the SMARCB1 dependent active enhancers. We identified 129 or 69 unique G401-B1 or G401 open acetylated regions associated differentially expressed genes. Of those, SMARCB1 unique genes are related to extracellular matriosome glycoproteins, muscle contraction, and epidermis development, i.e IGFBP3. GFP unique genes are associated with skeletal system development, mesoderm development, and neuron differentiation, i.e BMP7. Re-induction of SMARCB1 unique repressed to de novo H3K27ac marked active regions associated genes are responsible for maintaining homeostasis.
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 active 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. The accessible active enhancer associated differentially expressed genes are related to cytokine expression and inflammatory response.
Conclusion: Our analysis revealed that re-expression of SMARCB1 chromatin accessibility genome-wide, redistributes active enhancer landscape and restores cell homeostasis and senescence. ARID1A maintain the accessibility landscape at active enhancers and differentially regulates cytokine and inflammatory response genes.

FIXING CHIP WITH NEXT-GENERATION SPIKE-IN CONTROLS

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We recently used SNAP-ChIP® to deliver an unsettling message: many widely cited commercial antibodies to the methyl states of histone H3 lysine 4 (H3K4) do not perform in Chromatin ImmunoPrecipitation (ChIP) as promised (Shah *et al* (2018) *Mol Cell* **72:**162). As such there is a need to identify truly capable reagents for histone post-translational modifications (PTMs), and critically re-evaluate their genomic distribution and regulatory functions.
Data will be presented from our recent testing of >250 commercial antibodies to lysine PTMs with the SNAP-ChIP **K-MetStat™** and **K-AcylStat™** nucleosome panels. This reveals the 'ChIP-grade antibody' specificity / efficiency problem to be pervasive (and many are thus to be avoided), although fit-for-purpose reagents do exist and are for the first time identifiable as such.
SNAP-ChIP K-MetStat: me0-1-2-3 for H3K4, H3K9, H3K27, H3K36 and H4K20
SNAP-ChIP K-AcylStat: unmodified or single acylations [acetylation / butrylation/ crotonylation] on H3 (K4ac, K9ac/bu/cr, K14ac, K18ac/bu/cr, K23ac, K27ac/bu/cr, H3K36ac), H4 (K5ac, K8ac, K12ac, K16ac, K20ac); or combinatorial acetylations (H3K27ac+S28ph, tetraAc-H3, tetraAc-H4, tetraAc-H2A)

TET2/3-DEPENDENCE OF VITAMIN C-INDUCED EPIGENOMIC ALTERATIONS IN AN ACUTE MYELOID LEUKEMIA MODEL

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Background: Neomorphic mutations in isocitrate dehydrogenase 1/2 (*IDH1/2*) and inactivating mutations in Ten Eleven Translocation dioxygenase 2 (*TET2*) are frequent and mutually exclusive events in *de novo* acute myeloid leukemia (AML). *IDH1/2* mutations drive epigenomic dysfunction through production of the oncometabolite R-2-hydroxyglutarate (R-2HG), which inhibits the cytosine demethylation activity of TET2 and causes a local hypermethylation phenotype. We have previously shown that vitamin C (vitC) induces epigenetic reprogramming and cellular differentiation through re-activation of TET2 in an engineered murine leukemia model that overexpresses (OE) the *HOXA9* oncogene and mutant IDH1^{R132H}. Although TET mutations in AML patients occur exclusively in the *TET2* gene, TET2 and TET3 are expressed at equally high levels in both our murine model and in *de novo* AML blasts. Based on the reported functional redundancy among TET enzymes, we hypothesize that the vitC-induced re-establishment of methylation homeostasis and differentiation in *IDH* mutant cells is also mediated through TET3.
Methods: In order to delineate the individual contributions of TET2 and TET3 to vitC-induced reprogramming, we inactivated TET2 (*TET2*^{KO}) and TET3 (*TET3*^{KO}) individually or in combination (*TET2*^{KO}/*TET3*^{KO}) in *HOXA9*^{OE}/*IDH1*^{R132H} murine bone marrow cells using CRISPR/Cas9 technology. These cells were treated daily with 0.345mM vitC or control over 20 days. Cell growth and differentiation were assessed using standardized cell counts, cytospins, and flow cytometry. After 15 and 72 hours of vitC- vs control treatment, we sequenced total RNA as well as (hydroxy)methyl-immunoprecipitated DNA (hmeDIP- and meDIP-seq). In order to identify enhancers and hypermethylated genomic regions these data were integrated with previously generated H3K27ac and H3K4me1 ChIP-seq data from vitC- and untreated *HOXA9*^{OE}/*IDH1*^{R132H} cells and meDIP-seq data from untreated non-leukemic HOXA9^{OE}/IDH1^{WT} cells.
Results: In response to vitC treatment, both *TET2*^{KO} and *TET3*^{KO} but not *TET2*^{KO}/*TET3*^{KO} *HOXA9*^{OE}/*IDH1*^{R132H} cells showed reduced proliferation and increased levels of myeloid surface markers (Mac1/Gr1). In line with this observation, enhancers that were hypermethylated in untreated *HOXA9*^{OE}/*IDH1*^{R132H} cells, showed an increase in 5hmC levels both in *TET2*^{KO} and *TET3*^{KO} after 15 hours of vitC treatment. After 72 hours, the gain in 5hmC signal was markedly enhanced at distal enhancers within 20kb of genes that are implicated in myeloid differentiation (hypergeometric test $p<1x10^{-10}$) and upregulated in matched RNA-seq data, including *Csf2ra*, *Elane*, and *Cebpe*. To examine functional dependencies of TET2 and TET3, we observed a set of 136 genes implicated in myeloid activation that were commonly down-regulated upon loss of either TET2 or TET3. Interestingly, we could also identify a set of genes that were specifically up-regulated only in the presence of both TETs following vitC-treatment. These include S100a8, S100a9, and Tifab, which are key mediators of Toll-like receptor signaling and have been associated with myelodysplastic syndromes.
Conclusion: Our findings suggest a model in which vitC activates both TET2 and TET3 to reprogram the enhancer landscape of *IDH* mutant leukemic cells to drive myeloid differentiation. Consistent with this we found that TET2 and TET3 share overlapping functions in maintaining a myeloid activation signature and that disruption of either TET2 or 3 is sufficient to disturb it.

CISPLATIN TREATMENT OF TESTICULAR CANCER INTRODUCES LONG-TERM CHANGES TO THE EPIGENOME, POSSIBLY ASSOCIATED WITH METABOLIC SYNDROME

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Introduction: Testicular cancer (TC) survival rates have increased over the last decades, largely due to the introduction of cisplatin-based chemotherapy (CBCT). CBCT is part of standard treatment of several cancers. In TC survivors, an increased risk of developing metabolic syndrome (MetS) is observed.
Aims: In the epigenome-wide association study we investigated if CBCT relates to epigenetic changes, and if epigenetic changes render individuals susceptible for developing MetS later in life.
Methods: We analyzed DNA methylation, using the MethylationEPICBeadChip, in samples collected ~16 years after treatment from 279 Norwegian TC survivors with known MetS status. Among the CBCT treated (n=176) and non-treated (n=103), 61 and 34 developed MetS, respectively. We used linear regression models to identify if i) CBCT results in epigenetic changes, and ii) epigenetic changes play a role in development of MetS. Then we investigated if these changes in i) and ii) link to genes, functional networks and pathways related to MetS.
Results: We identified 35 sites that were differentially methylated when comparing CBCT treated and untreated TC survivors. The PTK6 - RAS - MAPk pathway was significantly enriched with these sites and infer a network of 13 genes with *CACNA1D* (involved in insulin release) as a network hub (gene with highest amount of interactions). We found nominal MetS-associations and a functional network with *ABCG1* and *NCF2* as network hubs. The *ABCG1* is involved in cholesterol and phospholipids transport and regulates lipid homeostasis. The NCF2 codes Neutrophil Cytosolic Factor 2, forms an enzyme complex called NADPH oxidase, which plays an essential role in the immune system.
Conclusion: Our results suggest that CBCT has long-term effects on the epigenome. Since we identified differential methylation occurring in genes associated with conditions pertaining to MetS,

we hypothesize that epigenomic changes may also play a role in development of MetS in TC survivors.

DELINEATING THE ROLE OF ENDOGENOUS RETROVIRUSES IN MELANOMA CELLS

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Endogenous retroviruses (ERVs) have colonized all metazoans. These elements are relics of retroviral infections that are fixed in the genomes of host species. These sequences possess the potential to dysregulate genes and pose pathogenic risks. Therefore, ERVs are generally repressed by host epigenetic systems. Intriguingly, a substet of elements have been co-opted to act as functional elements in host genomes, in a mechanism termed exaptation. It has been reported that some dysregulated ERVs function as alternative promotor or enhancer specifically in cancer tissues, which might contribute to the tumor development. However, the scope and mechanism of this phenomenon remains unclear.
In this study, we employed human melanoma cancer cell lines as models to delineate the function of ERVs through a combination of different epigenomic assays. We found that the dysregulation of specific subfamily of ERVs are associated with transcriptional changes in melanoma. These sequences potentially function as *cis*-regulatory elements in melanoma and are enriched with carcinoma process gene ontology terms. Moreover, we discovered some melanoma-specific novel bi-directional transcripts eminating from ERVs. The molecular functions of these ERV elements were analyzed by by CRISPR-Cas9 mediated deletion. Taken together, we aim to delineate the role of ERVs in regulating the epigenome and transcriptome of melanoma cells.

HISTRADER: A COMPUTATIONAL TOOL TO IDENTIFY TRANSCRIPTION FACTORS ENRICHED WITHIN THE NUCLEOSOME FREE REGIONS OF REGULATORY ELEMENTS IDENTIFIED USING CHIP-SEQ AGAINST HISTONE MODIFICATIONS.

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Chromatin immuno-precipitation sequencing (ChIP-Seq) against histone modifications is often used to identify changes to the epigenetic landscape that accompany cell fate decisions and the development of diseases such as cancer. Many of the changes to the chromatin landscape, alternative enhancer and promoter usage, are driven by the acquired or lost activity of transcription factors (TFs). *De novo* motif elucidation and enrichment analyses are often employed to identify the TFs responsible for the observed epigenomic changes. However, the putative regulatory elements identified by ChIP-Seq against histone modification are typically span several kilobases in length, which complicates motif-based analyses. Nevertheless, the ChIP-Seq signal profile of histone modifications display local minima and maxima that correspond to nucleosome free regions (NFRs), bound by TFs, and the nucleosomes harboring the modified histones, respectively. We developed and implemented HisTrader (Histone Trader) a computational tool to identify NFRs and extract the corresponding DNA sequences for motif enrichment analyses (MEA) to identify TFs occupying these regulatory elements. We validate our approach using publicly available data from the Encyclopedia or DNA Elements (ENCODE) project and a publicly available dataset of matched gastro-esophageal adenocarcinomas (GEA) profiled by ChIP-Seq. HisTrader accurately calls NFRs from histone modification ChIP-seq, which we validate by demonstrating their co-occurrence with open DNaseI hypersensitivity sites (DHS). In addition, HisTrader reveals that multiple NFRs often occur within individual regulatory elements identified by ChIP-Seq. Finally, Histrader can be used together with MEA to identify aberrantly expressed oncogenic or inactivated tumour-suppressing transcription factors within somatically gained or lost regulatory elements in GEA. In conclusion, HisTrader is a sensitive computational tool to discover NFRs with regulatory elements that can be implemented with existing tools to identify TFs involved in aberrant enhancer usage and gene expression changes observed in cancer.

EPIGENETIC MODIFICATIONS OF TYPE 2 DIABETES IN A MIDDLE EASTERN POPULATION

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T2D has a high prevalence in the Middle East and is a focus of many funded research studies in Qatar. In this study we aim to identify DNA methylation sites associated with T2D in the Qatari population. We use a large cohort of more than 800 samples and Illumina EPIC arrays for a wide coverage of CpG sites.
More than 800 DNA samples were obtained from Qatar Biobank, with 45% T2D patients selected based on a strict selection criteria. These were then profiled for DNA methylation using illumina EPIC arrays that measures more than 850,000 CpG sites. This cohort was split into a discovery cohort of 458 samples and a replication cohort of 381 samples. The minfi package in R statistical package was used to analyze the methylation data. Quality control steps involved removing bad samples, removing samples or CpG sites that didn't pass a detection p value, and removing CpG sites that overlapped with SNPs. In total, 835,000 CpG sites remained for analysis.
A linear regression model was used to find association between CpG sites and T2D, correcting for BMI, gender, cell counts, batch effects and well position. Smoking was not present for all samples and AHRR was used as a surrogate for smoking. Age showed a high collinearity with T2D and thus the CpG levels were corrected for age using control samples before using the regression model. A total of 2900 CpG sites were identified to be associated with T2D at a Bonferroni p value ($p < 5.8 \times 10^{-8}$) in the discovery cohort, among which 150 sites replicated in the replication cohort. Moreover, more than 20 known T2D DNA methylation loci were replicated in the discovery cohort at $p<10^{-4}$. Some of the identified loci are also in known T2D GWAS genes. We are

currently investigating all the sites and their biological pathways in T2D.
This study is by far the largest one done on T2D using the high coverage of EPIC technology, and also the first of its type in the Middle East. It revealed much more of the methylation sites involved in T2D pathways in general and in a Middle Eastern population in specific. We believe that such study may contribute to understanding the mechanisms underlying T2D.

GENOME-WIDE CHARACTERIZATION OF DNAm IN HUMAN PLACENTAL CELL TYPES

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INTRODUCTION: DNA methylation (DNAm) studies of the placenta have revealed striking epigenetic features that no other tissues exhibit. However, because most studies have focused on whole placental tissue samples, it remains unknown if these features are present in all constituent cell types or are confined to specific ones.
We hypothesize that DNAm is highly specific between placental cell types, and that canonical placental epigenetic features may be confined to specific constituent cell types.
METHODS: Placental cell types (trophoblasts, vascular endothelial cells, stromal fibroblast cells, and Hofbauer cells) were isolated from placental chorionic villi by fluorescence-activated cell sorting. Samples were collected from 9 first trimester (5 female), and 19 term (10 female) pregnancies. DNAm was measured using the Illumina MethylationEPIC array (>850 000 CpGs). After data processing, cell-specific differentially methylated CpGs (DMCs) were identified by linear modelling (Bonferroni-adjusted, $p<0.01$, effect size>0.25). Fisher's exact test was used to test for enrichment at various genomic elements at a false discovery rate of $p<0.01$.
RESULTS: Trophoblast cells were the most distinct cell type across gestation, with >110 000 DMCs detected for both first and third trimester comparisons. All cell-specific DMCs were over-represented in enhancer regions (FDR<0.01, odds ratio>1.6); and many DMCs localized to genes that regulate DNAm, e.g. 6/6 CpGs covering the CpG island of DNMT1, and 6/9 CpGs in the CpG shore of TET1. A high proportion of CpGs located in FLT1, which plays an important role in angiogenesis, were also cell-specific (44/77 of all FLT1 CpGs). Among CpGs mapping to placental-specific partially methylated domains (PMD), there was a higher proportion (79.6-82.8%) of partially methylated CpGs (<70% methylated) in trophoblast and whole placental villi, compared to other cell types (30.4-62.1%). Intermediate levels of methylation at placental-specific imprinted regions was more common for trophoblasts (63-68%) than other cell types (13-60%). For canonical general imprinted regions, we observed no tissue-specificity: the majority (78-87%) of CpGs were intermediately methylated for all cell types.
CONCLUSION: Trophoblast cells have the most unique DNAm profile compared to other cell types in placenta. Placental cell-specific DNAm is involved in developmental processes, based on enrichment for cell-specific DMCs in important regulatory regions and genes. Placental-specific DNAm features, such as PMDs and placental-specific imprinting, can be primarily attributed to DNAm in trophoblast cells rather than other constituent cell types.

FUNCTIONAL ANNOTATION OF GWAS REGULATORY VARIANTS POWERED BY ENSEMBL

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Functional interpretation of genome wide association studies (GWAS) remains a significant hurdle. Associated variants are generally not likely coding variants, rather potential regulatory variants with weak phenotypic associations. Here, we present how weak associations with causal genes can be detected through a genome-wide and multi-layered integrative analysis.
Ensembl's Regulatory Build synthesizes epigenomic datasets produced by large-scale projects such as ENCODE, Roadmap Epigenomics or BLUEPRINT. The resulting regulatory annotation defines biochemically active regions across 118 human cell types and 79 mouse cell types. To support the Regulatory Build, we maintain the International Human Epigenome Consortium's (IHEC) Epigenome Reference Registry (EpiRR), where large epigenomic consortia collect their metadata. To gain further insight, Ensembl is developing a database of cis-regulatory interactions that attach them to their target genes; as a first step all of the GTEx summary eQTL data is incorporated and can be accessed and viewed.
Having brought all this data together, we demonstrate the possibilities of functional data integration with our post-GWAS analysis platform. This algorithm compares human GWAS results, as stored in public archives or in a private individual study, to a collection of genomic annotations, many of which are stored in Ensembl, producing a list of putative causal genes along with linked evidence. It optionally integrates a Bayesian colocalization algorithm that stochastically tests possible sets of causal variants at each GWAS peak. This pipeline can be quickly deployed locally and will also soon be available online on the Ensembl genome browser.

THE THREE-DIMENSIONAL GENOME ATLAS OF PRIMARY PROSTATE CANCER

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The human genome is organized through hierarchical chromatin loop interactions in three-dimensional (3D) space to ensure tightly regulated gene expression programs. Genetic alterations targeting chromatin loop interactions have conceivably been reported to drive aberrant gene expression in cancer. Despite advances in chromatin conformation capture technologies however, majority of studies to-date focus on cell lines and liquid tumors largely due to sample inaccessibility and insufficient input. Overcoming these challenges, we conducted Hi-C on 10-µm thick cryosections harvested from 13 primary prostate tumors (i.e 13 patients) with matching H3K27ac ChIP-seq, whole-genome sequencing (WGS), RNA-seq and proteomics. Upon sequencing, we clearly see the interaction between the TMPRSS2 and ERG loci in 6/13 tumors that harbor the genetic fusion as expected from our previous WGS findings. Confident in our dataset, we delineated the hierarchical 3D organization of chromatin loops for each tumour and find more inter-patient differences at smaller kilobase-scale chromatin loops compared to larger megabase-scale chromatin loops between the 13 patients. Motivated by the frequent presence of structural variants (SVs) in prostate cancer, we also used our Hi-C maps and identified a total of 290 SVs across the thirteen patients (6-77 at the per patient level). While we identified numerous SVs previously reported through WGS such chr3:129-130 Mb SVs, we discovered a plethora of novel intra- and inter- chromosomal SVs. Our results suggest that at least a part of the inter-patient 3D organization and gene expression differences can be attributed to the SVs. Taken together, our work mapped out the 3D primary prostate cancer atlas and identified novel SVs with functional consequences.

EPIGENETIC REPROGRAMMING IN A HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA MODEL

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Background: T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive blood cancer of malignantly transformed immature thymocytes. While epigenetic reprogramming has been implicated in the initiation of T-ALL the lack of an *in vitro* model and appropriate normal controls has hampered the quantitative measurements of epigenetic signatures associated with T-ALL. To address the hypothesis that epigenetic alterations are contributing initiating events in T-ALL we have performed temporal epigenetic and transcriptome profiling of a novel human T-ALL model and untransformed controls.
Methods: Cord blood derived CD34+ (CB CD34+) cells were transduced with a constitutively active NOTCH1 allele, LMO2, TAL1 and BMI1 (NLTB) and cultured *in vitro* under conditions that promote T-cell differentiation. The co-cultured but non-transduced CB cells served as the negative control. Three time points following transfection (Day 14, 24 and 47) were collected for epigenetic (histone ChIP-seq) and transcriptome (RNA-seq) profiling.
Results: Hierarchical clustering of protein coding gene expression, H3K27me3 and H3K4me3 promoter density separated NLTB transduced from non-transduced CD34+ cells regardless of the *in vitro* collection day. In comparison to parental CD34+ cells profiled prior to *in vitro* culturing, both of the transduced and non-transduced CD34+ cells showed directional increases in the genomic space occupied by H3K4me3. Furthermore, the NLTB transduced CB CD34+ cells showed increased genomic H3K4me3 occupancy compared to non-transduced normal comparators, the directionality of which was observed consistently across all three time points. Surprisingly, unique genomic regions consistently marked by H3K4me3 in the NLTB transformed CB CD34+ cells were associated with gene bodies and intergenic regions rather than gene promoters as might be expected for this mark. Enrichment analysis of genes containing genomic regions uniquely marked by H3K4me3 in NLTB transformed CB CD34+ cells were significantly enriched in terms relevant to leukemic biology. A subset of uniquely H3K4me3-marked regions were co-localized with H3K27ac peaks, enriched in ETS and RUNX binding motifs and were associated with non-coding transcripts, implying regulatory roles or aberrant expression of lncRNA. Similar directionality was observed in H3K27me3 occupancy in NLTB transformed CB CD34+ cells vs. their non-transduced controls. Integrative analysis of epigenetic and transcriptional alterations associated with T-ALL transformation allowed for the identification of putative targets of the transduced oncogenes, such as MYCN.
Conclusion: The dynamic epigenetic reprogramming at T-ALL

initiation, as revealed by this study, helped dissect the collaborative effects associated with NOTCH1, LMO2, TAL1, and BMI1, providing novel therapeutic targets for therapy tailored to TAL/LMO subtype of T-ALL.

IMPACT OF PHENOTYPIC SEX AND SEX CHROMOSOME COMPLEMENT ON SHAPING DNA METHYLATION IN MOUSE LIVER

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Male and female mammals have substantial differences in phenotype and gene expression. A complex regulatory network, which includes sex-biased patterns of DNA methylation, has been previously shown to be behind the observed differences in gene expression between the two sex phenotypes. We carried out a survey of mouse liver DNA using Whole Genome Bisulfite Sequencing (WGBS) to detect sex-associated Differentially Methylated Regions (sDMRs) and understand the role played by both gonadal hormones and sex chromosomes in the establishment of DNA methylation in males and females. The WGBS data was obtained from mice with different combinations of sex chromosome complements and phenotypic sex: 5 XX females (XXf), 3 XY males (XYm), 3 XY females (XYf), and 3 XO females (XOf).

After CpG methylation calling, sDMRs were detected by doing pairwise comparisons between the experimental groups (e.g. XXf vs XYm, XYf vs XYm, XOf vs XXf, and XXf vs XYf) using two alternative packages: MethylKit and DSS. A total of 603 sDMRs were detected in more than one comparison. Of those, 587 were found only in comparisons across different sex phenotypes, whereas 16 were found only in comparisons with different sex chromosome complements, suggesting that gonadal sex is an important contributing factor for sex-associated differences in DNA methylation, while chromosome load plays only a complementary role.

Finally, a motif enrichment analysis was performed on the 587 sDMRs detected in groups with different sex phenotypes. Significantly enriched motifs were identified, including several associated with transcription factor binding sites such as: CUX2, HNF6, and STAT5. These transcription factors have been previously linked with the establishment of sex-biased gene expression in mouse liver. Interestingly, in our results, we also observe sDMRs in proximity to the *Cux2, Hnf6*, and *Stat5b* genes themselves, suggesting a potential regulatory feedback loop. In conclusion, differential methylation analysis and the subsequent motif enrichment detection provide clues on the mechanisms underlying sexual dimorphism in phenotypes.

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