CEEHRC NETWORK
Canadian Epigenetics, Environment and Health Research Consortium Network

IHEC
International Human Epigenome Consortium

JOINT ANNUAL MEETING

4–7 October 2022

Estérel Resort, Estérel, Québec, Canada

CEEHRC

Canadian Epigenetics and Environment Health Research Consortium

IHEC

International Human Epigenome Consortium

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

ESTĒREL RESORT

Dear Colleagues,

It is a pleasure to welcome you to Estérel Resort in beautiful Estérel, Québec for the 8th Annual Canadian Epigenetics and Environment Health Research Consortium Epigenetic Conference being held in partnership with the International Human Epigenome Consortium Annual General Meeting. I am grateful for the opportunity to gather and learn together on the traditional, ancestral, and unceded territory of the Mohawk and Atikamekw peoples who for millennia have passed on their culture, history, and traditions from one generation to the next on this site.

This year we welcome over 250 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 12 sessions that include keynote and plenary lectures, technical talks, rapid fire oral presentations, a workshop on IHEC integrative analysis resources and data portal, 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.

I would like to take this opportunity to highlight the CEEHRC Trainee Committee who continue to make significant and impactful contributions to the CEEHRC Network. I encourage you to engage and share their outstanding work that is at the foundation of the outreach initiative launched at the 6th Annual Meeting, providing evidence-based interpretations of epigenetics research and its implications. The broader 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. More information about the Network and its goals can be found at our website: www.thisisepigenetics.ca.

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

Sincerely,

Martin Hirst, PhD
Distinguished Scientist, BC Cancer Research Institute
Director, Michael Smith Laboratories
Professor, Department of Microbiology and Immunology

Table of Contents

Organizing Committee


Sinéad Aherne
BC Cancer


Steve Bilodeau
Université Laval


Guillaume Bourque
McGill University


Julie Brind'amour
Université de Montreal


Carolyn Brown
University of British Columbia


Genevieve Deblois
Université de Montreal


Elizabeth Elder
Université de Montreal


Marco Gallo
University of Calgary


Martin Hirst
University of British Columbia


Maja Jagodic
Karolinska Institute


Sarah Kimmins
Université de Montréal


Tony Kwan
McGill University


Mathieu Lupien
University of Toronto


Eric Marcotte
CIHR


Serge McGraw
Université de Montréal


Dena Procaccini
NIH


Toshikazu Ushijima
National Cancer Center Research Insitute, Japan


Jorn Walter
Saarland University

Keynote Speakers

Nada Jabado
McGill University

Dr. Jabado began her career as an independent investigator in 2003 at the RI-MUHC, pioneering a research program in pediatric brain tumors, which is now unparalleled. Dr. Jabado's group was among the first to identify a histone mutation in human disease which has revolutionized this field. The epigenome was a previously unsuspected hallmark of oncogenesis and this discovery linked development and what we now know are epigenetic-driven cancers. Dr. Nada Jabado has over 150 peer-reviewed publications to her credit in such prominent journals as Nature Genetics, Nature, Science and Cancer Cell. She is invited as keynote speaker to International symposia and top ranked universities and has been awarded funding from CIHR, Genome Canada, NIH, as well as a Large-Scale Genomics grant from Genome Canada. In 2015, Dr. Jabado was named as a Fellow of the Royal Society of Canada in the Life Sciences Division.

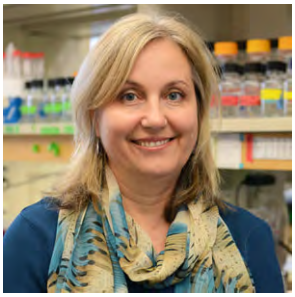
Jamie Hackett
EMBL

Jamie Hackett is a group leader at the European Molecular Biology Laboratory (EMBL), within the Epigenetics and Neurobiology unit in Rome, Italy. He obtained his PhD at the University of Edinburgh, and completed postdoctoral training at the University of Cambridge, UK, under Prof. Azim Surani. Jamie's research group has two overarching scientific themes. (i) To dissect the regulatory impact and logic of chromatin changes in health and disease, by engineering precision epigenome manipulations. (ii) To investigate the potential for (altered) epigenetic states to be transmitted through mitosis or meiosis and influence phenotype.

Invited Speakers

Cheryl Arrowsmith
University of Toronto

Dr. Arrowsmith received her Ph.D. in chemistry from the University of Toronto and carried out postdoctoral research at Stanford University. She is the director of the Toronto Node of the Structural Genomics Consortium (SGC), Senior Scientist at the Princess Margaret Cancer Centre, and Professor of Medical Biophysics at the University of Toronto. Dr. Arrowsmith is an internationally recognized expert in cancer related structural biology & chemical biology and epigenetics. She has been the leader of the Toronto site of the SGC since its inception and coordinates the SGC's epigenetic chemical probe program. Dr. Arrowsmith was co-founder of Affinium Pharmaceuticals, a structure-based biotech that developed an anti-MRSA antibiotic that recently completed successful phase IIa trials. She has served as a member of 18 scientific advisory boards, grant review panels and journal editorial boards since 2000, including current membership on the Board of Directors of Academic Drug Discovery Consortium (ADDC). Dr. Arrowsmith holds a Canada Research Chair in Structural Genomics at the University of Toronto, has published over 250 peer reviewed articles, with more than 60 of these published in the last 5 years, and is co-author of over 1800 3D protein structures in the Protein DataBank (PDB). Her current research focuses on structural biology and chemical biology approaches to understand how transcriptional, and chromatin regulatory proteins recognize, interact with and signal to other molecular components of the cell.



Nathalie Berube
University of Western Ontario

Dr. Bérubé is a Professor in the Departments of Paediatrics, Anatomy and Cell Biology, and Oncology at Western University and a Scientist at the Children's Health Research Institute in London, Canada. Her research intersects neuroscience and epigenetics with a focus on the role of chromatin regulators in learning and memory and in neurodevelopmental disorders. Using mouse genetics, her group is investigating the function of the ATRX chromatin remodeling protein in brain development, and in specific cell types of the central nervous system. The methods used include the culture of primary cells, genome-wide sequencing, bioinformatics, and behavioral testing to better understand the effects of ATRX deficiency in the brain.

Guillaume Bourque
McGill University

Guillaume Bourque is a Professor in the Department of Human Genetics at McGill University and the Director of Bioinformatics at the McGill University Genome Center. He is a member of the Research Advisory Board of CIHR's Institute of Genetics, of the Research Advisory Council of Compute Canada, the national platform for high-performance computing, of CANARIE, responsible for Canada's ultra-fast network backbone and on the External Consultant Panel of ENCODE. He leads the Canadian Center for Computational Genomics (C3G), a Genome Canada bioinformatics platform, and the McGill initiative in Computational Medicine (MiCM). He is also the head of the Epigenomics Mapping Center at McGill, a project that oversees data generation and processing as part of the Canadian Epigenetics, Environment and Health Research Consortium (CEEHRC). His research interests are in comparative and functional genomics with a special emphasis on applications of next-generation sequencing technologies.

Melanie Eckersley-Maslin
University of Melbourne

Dr Melanie Eckersley-Maslin is research group leader and Snow Medical Fellow at the Peter MacCallum Cancer Centre and a research fellow in Anatomy and Physiology at the University of Melbourne, Australia. Her laboratory investigates the role and regulation of epigenetic plasticity in facilitating cell fate changes in both developmental and cancer models using single-cell and CRISPR-based technologies. Melanie completed her PhD in molecular biology at Cold Spring Harbor's School of Biological Sciences in New York, USA with Professor David Spector. In 2014, she moved to the Babraham Institute, Cambridge UK to work with Prof Wolf Reik as a postdoctoral research fellow supported by an EMBO Fellowship, Marie Curie Independent Fellowship and a BBSRC Discovery Grant. There she made seminal discoveries on the epigenetic control of early embryonic cell fate transitions including zygotic genome activation and gastrulation and discovered some of the first epigenetic priming factors in early development. In 2021 she returned to Australia to establish her laboratory at Peter Mac combining her interests and background in developmental epigenetics to get new insights and perspectives into cancer evolution and adaptation, with the ultimate aim to identify new prognostic markers and therapeutic targets. She was recently awarded the 2020 Metcalf prize for Stem Cell research by the National Stem Cell Foundation of Australia, and the prestigious 8-year Snow Medical Fellowship in 2021.

Sarah Kimmins
Université de Montréal

Sarah Kimmins is a Professor at the Université de Montréal in the Department of Pathology and Cellular Biology, an affiliate member of Pharmacology and Therapeutics, at McGill University, Canada, and was the Associate Director of the McGill Center for Research in Reproduction and Development (2014-2017). She is the current Chair of the Andrology Special Interest group for the Canadian Fertility and Andrology Society and a member of the International working group on Mens' Reproductive Health. She was awarded a tier 1 Canada Research Chair in Epigenomics, Reproduction and Development in 2021and held a tier 2 CRC from 2011-2020. She received the Society for the Study of Reproduction Young Investigator award and the American Society of Andrology Young Andrologist Award. Her research team is focused on understanding the molecular mechanisms underpinning paternal epigenetic inheritance. Particular interest is given to paternal health and how environmental exposures (diet, BMI, toxicants) alter fertility, clinical outcomes, the sperm and embryo epigenome, development and health of offspring.

Julie Lessard
Institut de recherche en immunologie et en cancérologie de l'Université de Montréal

Julie Lessard obtained her Ph.D. in Molecular Biology in 2003 following research work supervised by Dr. Guy Sauvageau at l'Institut de recherches cliniques de Montréal (IRCM). She then left for California to carry out postdoctoral training at Stanford University, in Gerald R. Crabtree's laboratory. She became familiar with the proteomics approach and enhanced her knowledge of epigenetic mechanisms that control stem cell function. She joined the IRIC team in 2007. Her group's recent work suggests that specialized assemblies of SWI/SNF complexes regulate cell fate determination in the hemopoietic tissue. Using quantitative proteomics and molecular genetics approaches, we identified subunits that are essential for hemopoietic stem cell (HSC) function and others that are required later in the hemopoietic hierarchy, for the development of specific blood-cell lineages. Most importantly, we identified a key component of the combinatorial code that dictates hemopoietic cell fates as being the mutually exclusive usage of the Smarcd1, Smarcd2 and Smarcd3 family subunits. Using conditional knock-out alleles, we showed that Smarcd1, d2 and d3 are master regulators of lymphoid, granulocytic and hemopoietic stem cell (HSC) development, respectively. Globally, our research program aims at characterizing the molecular basis for lineage determination that emerges from combinatorial assembly of alternative subunits within hemopoietic SWI/SNF complexes.

Matthew Lorincz
University of British Columbia

Dr. Lorincz received a BA in the Department of Zoology at the University of California, Berkeley, and did his PhD thesis work under the supervision of Dr. Leonard Herzenberg in the Department of Genetics at Stanford University. From 1999-2005 he was a Postdoctoral Fellow and subsequently Staff Scientist at the Fred Hutchinson Cancer Research Center under the supervision of Dr. Mark Groudine. He took a faculty position at the University of British Columbia in 2005, where he is currently a Full Professor and Interim Head in the Department of Medical Genetics. Research in the Lorincz lab focusses on dissecting the interplay between covalent histone modifications, DNA methylation and transcription in embryonic and germline development, using the mouse as a model system.

Alexander Meissner
Max Planck Institute for Molecular Genetics

Alexander Meissner studied Medical Biotechnology at the Technical University Berlin before starting his PhD studies with Rudolf Jaenisch at the Whitehead Institute/MIT in 2002. He completed his PhD in 2006 and spent the next year and a half working with Rudolf Jaenisch and Eric Lander before starting his own lab as an assistant professor in the Department of Stem Cell and Regenerative Biology at Harvard University and as an associate member of the Broad Institute in 2008. He was promoted to associate professor in 2012 and full professor with tenure in 2015. In 2016 he has been appointed as Director and Head of the Department of Genome regulation at the Max Planck Institute for Molecular Genetics in secondary employment and changed it to his principal employment in 2017.

Jennifer Mitchell
University of Toronto

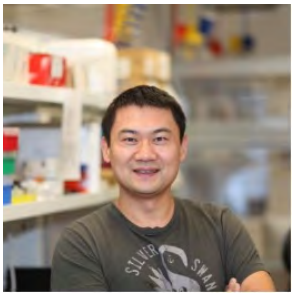
Dr Mitchell is an associate professor at the University of Toronto in the Department of Cell and Systems Biology where she uses CRISPR genome engineering to study the mechanisms through which transcriptional regulatory elements activate gene expression in stem cells. Dr Mitchell received her PhD from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

Will Pastor
McGill University

William Pastor earned his Ph.D. at Harvard University in the lab of Dr. Anjana Rao. He was part of the team that discovered that Tet enzymes oxidize the epigenetic mark 5mC to form 5hmC. He subsequently invented a method to map 5hmC in the genome. As a postdoctoral fellow in the labs of Dr. Steven Jacobsen and Dr. Amander Clark at UCLA, he studied mechanisms of 5mC establishment and transcriptional regulation in germ and stem cells. He became an Assistant Professor at McGill University in 2018. His lab developed a stem cell model of epigenome acquisition in placental lineage and presently studies transcriptional regulation and 5mC patterning during early embryonic and placental development.

Gustavo Turecki
McGill University

Gustavo Turecki is a clinician-scientist who has been involved in the investigation of the neurobiology of depression and suicide, with a particular interest on functional genomics and epigenetics. Dr Turecki's laboratory has made important contributions to our understanding of biological processes underlying psychopathology, including the first description of molecular mechanisms explaining the impact of childhood traumatic experiences on brain function. Dr. Turecki's work uses epidemiological, clinical, psychosocial and basic research approaches, and has significantly advanced knowledge on mechanisms leading to suicide and suicidal behaviour. In addition, his laboratory has also made important contributions to the investigation of mechanisms of antidepressant response.



Ting Wang
Washington University School of Medicine

Dr. Ting Wang is the inaugural Sanford C. and Karen P. Loewentheil Distinguished Professor of Medicine at Washington University School of Medicine in St. Louis. Dr. Wang is recognized for his research on genetic and epigenetic impact of transposable element (TE) on gene regulation. His group is known for defining the widespread contribution of TEs to the evolution of species-specific gene regulatory networks as well as to the conservation of 3D genome architecture, and for revealing that epigenetic dysregulation of TEs is a major mechanism driving oncogenesis. Dr. Wang's lab investigates epigenetic determinants of cell fates in normal development and regeneration, in cancer, and in evolution, by integrating cutting-edge experimental and computational technologies. His lab developed DNA methylomics technologies, algorithms to identify regulatory motifs and modules, and analytical and visualization tools to integrate large genomic and epigenomic data. His lab is home to the WashU Epigenome Browser, utilized by investigators around the world to access hundreds of thousands of genomic datasets generated by large Consortia including the NIH Roadmap Epigenome Project, ENCODE, 4D Nucleome, TaRGET, IGVF, and the Human Pangenome Project. Dr. Wang currently directs the NIEHS Environmental Epigenomics Data Center, the Human Pangenome Reference Consortium, and the IGVF Data Administrative and Coordination Center.

Japan-Canada International Collaborations

David Langlais
McGill University

David Langlais is an Assistant Professor of Human Genetics and Microbiology & Immunology at McGill University, and Principal Investigator at the McGill Genome Centre. Dr Langlais completed his PhD with Dr Jacques Drouin at the IRCM. Dr Langlais then pursued postdoctoral studies with Dr Philippe Gros at McGill University on the role of critical innate immunity transcription factors and participated in the characterization of new proteins involved in immune function and neuroinflammatory conditions, including cerebral malaria. His lab studies the role of various transcriptional regulators in normal immune responses and in infectious and chronic inflammatory diseases.

Jay Shin
*Genome Institute of Singapore (GIS) A*STAR*

Dr. Jay W Shin acquired his PhD at the ETH Zürich, Switzerland after his research training at Harvard Medical School (HMS), Boston, USA. From 2008, Jay joined RIKEN Yokohama, Japan after receiving a prestigious Special Postdoctoral Research Fellowship and later became a Principal Investigator where he led a diverse group of research teams including FANTOM and Human Cell Atlas and partnered with world-leading industries related to stem cells and non-coding regulatory RNAs. Jay recently joined Genome Institute of Singapore (GIS) A*STAR to initiate a new program on Regulatory Genomics and develop pioneering technologies, including single cell genomics and large-scale molecular phenotyping platforms, to benefit both basic and applied sciences.

Soichiro Yamanaka
Tokyo University

Soichiro Yamanaka received his PhD from The University of Tokyo in 2010, then continued his studies as a Visiting Scientist in National Institutes of Health. In 2013, he was appointed Assistant Professor, Keio University School of Medicine, and since 2019, has been an Associate Professor at The University of Tokyo.

Ahmed Ashraf
University of Manitoba

Ahmed Ashraf received his PhD from Carnegie Mellon University in Electrical and Computer Engineering in 2010. He then continued postdoctoral studies at the University of Pennsylvania and at the Toronto Rehabilitation Institute. Since 2018, Dr. Ashraf has been an Assistant Professor at the Unversity of Manitoba where his interests lie in research interests include Artificial Intelligence, Machine Learning, Robotics, and Computer Vision.

Akihide Yoshimi
National Cancer Center Research Institute

I am a physician scientist and have been a PI as a section head and currently a chief of Div. Cancer RNA Research at National Cancer Center Japan. Our lab is interested in how aberrant splicing drives carcinogenesis and how it can be targeted. Current exciting collaboration with my friend Dr. Ly Vu at Simon Fraser University is especially focused on a potential interaction between global alterations in splicing, epigenome and epitranscriptome in leukemias and other tumors.

Ly Vu
University of British Columbia

Dr. Vu is a Scientist at the Terry Fox Laboratory, BC Cancer and an Assistant Professor at Faculty of Pharmaceutical Sciences at University of British Columbia (UBC). Dr. Vu's laboratory aims to understand molecular mechanisms underlying control of stem cells and pathogenesis of hematological malignancies with a focus on post-transcriptional gene regulation pathways, in particular RNA modifications. The ultimate goal is to develop innovative therapeutic approaches for treatment of leukemia and other cancers.

Epigenetic Technologies

Adriana Suarez
Sr. Science & Technology Advisor
10x Genomics

Hussein Daoud
Senior Genomics and Sequencing Sales Specialist
Illumina

Mrinal Pal
Field Application Scientist
New England Biolabs

Eric Leblond
Country Manager - Canada
Olink

Abstract Selected Speakers

Geraldine Delbes
Associate Professor
Institut national de la recherche scientifique (INRS)

Kinjal Desai
Research Associate
Hospital for Sick Children

Sarah Grasedieck
Postdoctoral Fellow
University of British Columbia

Cristian Groza
PhD Candidate
McGill University

Michael Johnston
Postdoctoral Fellow
University of Calgary

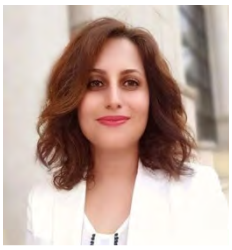
David Labbé
Assistant Professor
McGill University

Kiran Nakka
Postdoctoral Fellow
Ottawa Hospital Research Institute

David Nie
PhD Candidate
University of Toronto

Daniel Rico Rodriguez
Group Leader
Newcastle University

Martin Sauvageau
Assistant Professor
Institut de recherches cliniques de Montréal



Parisa Shooshtari
Assistant Professor
Western University

Michael Wilson
Principal Investigator
Hospital for Sick Children

Rapid Fire Talks

Jose Mauricio Ayala Esparza
PhD Candidate
McGill University
Supervisor: David Langlais

Cheuk Yin Cheng
PhD Candidate
Hong Kong University of Science & Technology
Supervisor: Danny Leung

Habib Daneshpajouh
PhD Candidate
Simon Fraser University
Supervisor: Maxwell W Libbrecht

Marina Fukano
PhD Candidate
Université de Montreal
McGill University
Supervisors: Genevieve Deblois & Morag Park

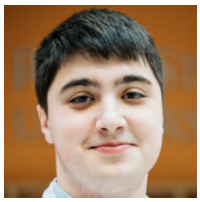
Amanda Ha
PhD Candidate
University of British Columbia
Supervisor: Louis Lefebvre

Axel Hauduc
PhD Candidate
University of British Columbia
Supervisor: Martin Hirst

Dennis Hecker
PhD Candidate
Goethe University
Supervisor: Marcel H. Schulz

Vrinda Mathur
Graduate Student
Hong Kong University of Science & Technology
Supervisor: Danny Leung

Anastasia Roemer
MSc Candidate
University of Alberta
Supervisor: Michael Hendzel



Dmitrijs Rots
PhD Candidate
Radboudumc
Supervisor: Tjitske Kleefstra

IHEC Business Day

Tuesday, October 4th, 2022

07:00 - 08:30	Breakfast *(Bistro à Champlain)*
GENERAL SESSIONS	
08:30 - 09:00	Introduction *Eric Marcotte*
09:00 – 09:30	Integrative Analysis Overview *Martin Hirst*
09:30 – 11:30	Data Analysis Dependencies and Metadata Discussion *All*
11:30 - 12:30	Lunch (*Bistro à Champlain*)
12:30 - 14:30	Flagship and Companion Papers *Figure Leaders (2-9)*
14:30 - 15:00	Coffee Break
15:00 - 16:00	IHEC – Lessons Learned *Mike Pazin* *Tomasz Dylag* *Danny Leung* *Charles Dupras*
16:00 - 16:15	Future Directions – Presentation *Martin Hirst*
16:15 - 17:00	Future Directions – Discussion *All*

IHEC Science Days / CEEHRC Agenda

Tuesday, October 4th, 2022

16:30 - 18:30	Registration *(Fridolin-Simard Lobby)*
KEYNOTE PRESENTATION I Introduction: Elizabeth Elder	
18:30 - 19:30	Jamie Hackett (EMBL) *Epigenetic Inheritance: From phenotypic variation to mechanisms*
19:30 - 21:30	Cocktail Reception *(Salon Dupuis)*

Wednesday, October 5th, 2022

07:00 - 07:55	Breakfast *(Bistro à Champlain)*
07:55 - 08:00	Opening Remarks - Martin Hirst
DNA Methylation Chair: Martin Hirst	
08:00 - 08:30	Alexander Meissner (Max Planck Institute for Molecular Genetics) *Epigenetic mechanisms in development and disease*
08:30 - 09:00	Matthew Lorincz (University of British Columbia) *Chromatin-guided DNA methylation: Insights from the mouse germline & early embryo*
09:00 - 09:15	Geraldine Delbes (Institut national de la recherche scientifique (INRS) *DNA methylation dynamic in male rat germ cells during gametogenesis*
09:15 - 09:30	David Nie (University of Toronto) *Discovery of a potent and selective degrader of the histone methyltransferase NSD2*
09:30 - 10:00	Coffee Break
Developmental Epigenetics I Chair: Serge McGraw	
10:00 - 10:30	Melanie Eckersley-Maslin (University of Melbourne) *Epigenetic plasticity in development and cancer*
10:30 - 11:00	Sarah Kimmins (Institution) *Environmental impacts on the heritable sperm epigenome*
11:00 - 11:15	Martin Sauvageau (Institut de recherches cliniques de Montréal) *Conserved role of the lncRNA CRNDE in regulating senescence and promoting colorectal cancer cell proliferation*
11:15 – 11:30	Cristian Groza (McGill University) *Genome graphs detect human polymorphisms in active epigenomic states during influenza infection*
11:30 - 13:00	Lunch and Learn *(ROK, Bistro à Champlain)*
Workshop: IHEC Data Portal and Integrative Analysis Moderators: Martin Hirst, Guillaume Bourque, David Bujold	
13:00 - 14:00	Introduction to the IHEC Data Portal and its large resource of reference epigenome datasets. We will show you the different types of datasets, in terms of assays and in cell and tissue types, and how to navigate the portal to publicly access and download selected data. We will also explore some of the integrative analysis being carried out on these datasets, and how this may be useful in your own research programs.
14:00 - 14:30	Coffee Break

Rapid-Fire Talks Chair: Mathieu Lupien	
14:30 – 14:35	Jose Mauricio Ayala Esparza (McGill University, PI: David Langlais) *Glutathione Metabolism Is a Regulator of the Acute Inflammatory Response of Monocytes to (1→3)-β-D-Glucan*
14:35 – 14:40	Cheuk Yin Cheng (Hong Kong University of Science & Technology, PI: Danny Leung) *Epigenomic Landscape of Transposable Element-driven Long non-coding RNAs in Hepatocellular Carcinoma*
14:40 – 14:45	Habib Daneshpajouh (Simon Fraser University, PI: Maxwell W Libbrecht) *Continuous chromatin state feature annotation of the human epigenome*
14:45 – 14:50	Marina Fukano (Universite de Montreal/McGill University, PI: Genevieve Deblois/Morag Park) *Investigating the Epigenetic Regulation of Intra-Tumour Metabolic Heterogeneity in Triple-Negative Breast Cancer*
14:50 – 14:55	Amanda Ha (University of British Columbia, PI: Louis Lefebre) *The Mest DMR regulates Klf14 imprinting via allele-specific sub-TAD structures*
14:55 – 15:00	Axel Hauduc (University of British Columbia, PI: Martin Hirst) *Cell-type-specific genetic-to-epigenetic relationships in the human breast*
15:00 – 15:05	Dennis Hecker (Goethe University, PI: Marcel Schulz) *The adapted Activity-By-Contact model for enhancer-gene assignment and its application to single-cell data*
15:05 – 15:10	Vrinda Mathur (Hong Kong University of Science & Technology, PI: Danny Leung) *The impact of SARS-CoV-2 on the maternal-fetal interface through single cell transcriptomics and epigenomics*
15:10 – 15:15	Anastasia Roemer (University of Alberta, PI: Michael Hendzel) *Mechanisms Governing the Accessibility of Dna Damage Proteins to Constitutive Heterochromatin*
15:15 – 15:20	Dmitrijs Rots (Radboudumc, PI: Tjitske Kleefstra) *KMT2C Haploinsufficiency Results in A Neurodevelopmental Disorder with Distinct Clinical Features And A Unique Dna Methylation Signature*
15:20 - 15:30	Coffee Break
KEYNOTE PRESENTATION II Introduction: Guillaume Bourque	
15:30 - 16:30	Nada Jabado (McGill University) *Deciphering Oncohistones pathogenesis in cancer and ... neurodegeneration*
18:30 - 20:00	Dinner *(Bistro à Champlain)*
Poster Session 1 (Salon Dupuis, Salle Esterel)	
20:00 - 22:00	See Poster Assignments (page 16)

Thursday, October 6th, 2022

07:00 - 08:00	Breakfast *(Bistro à Champlain)*
Developmental Epigenetics II Chair: Julie Brind'Amour	
08:30 – 09:00	Ting Wang (Washington University School of Medicine) *Dark matters in the epigenome*
09:00 - 09:30	Jennifer Mitchell (University of Toronto) *CRISPR deletion provides mechanistic insight into gene expression regulation by distal enhancers*
09:30 - 09:45	Parisa Shooshtari (Western University) *Integrating Chromatin Accessibility Data with the Data from Genome-Wide Association Studies Uncovers Transcription Factors Involved in Nine Autoimmune Diseases*
09:45 – 10:00	Kiran Nakka (Ottawa Hospital Research Institute) *Non-redundant regulatory role of H3K27me3 demethylases JMJD3 and UTX unveil pro-regenerative function of hyaluronan in muscle regeneration*

10:00 - 10:30	Coffee Break
Epigenetic Technologies Chair: Steve Bilodeau	
10:30 – 10:45	Adriana Suarez (10x Genomics) *Trailblazing the future of single cell and spatial biology*
10:45 - 11:00	Hussein Daoud (Illumina) *Leverage Illumina innovation to unlock your research potential*
11:00 - 11:15	Mrinal Pal (New England Biolabs) *NEBNext® EM-seq and CST CUT&RUN: High-performance alternatives for epigentic research*
11:15 - 11:30	Eric Leblond (Olink) *Precision proteomics broadens MultiOmics repertoire and enables biomarker discovery*
11:30 - 12:30	Lunch
Neuroepigenetics Chair: Marco Gallo	
12:30 - 13:00	Nathalie Berube (University of Western Ontario) *Linking cell type-specific chromatin dysregulation to memory deficits in mice*
13:00 - 13:30	Gustavo Turecki (McGill University) *The depressed brain at single-cell resolution*
13:30 - 13:45	Kinjal Desai (Hospital for Sick Children) *OLIG2 mediates a rare targetable stem cell fate transition in sonic hedgehog medulloblastoma*
13:45 - 14:00	Michael Johnston (University of Calgary) *Type B Ultra Long-Range Interactions in PFAs (TULIPs) are recurrent epigenomic features of PFA ependymoma*
14:00 - 14:30	Coffee Break
Transcriptional Regulation Chair: Genevieve Deblois	
14:30 - 15:00	Cheryl Arrowsmith (University of Toronto) *New tools for pharmacological modulation of the epigenome and transcriptome*
15:00 - 15:30	Julie Lessard (Institut de recherche en immunologie et en cancérologie) *Lineage-Specific Roles of SWI/SNF Chromatin Remodeling Complexes in Hemopoiesis*
15:30 - 15:45	Daniel Rico Rodriguez (Newcastle University) *Esearch3D: Propagating gene expression in chromatin networks to illuminate active enhancers*
15:45 - 16:00	Michael Wilson (Hospital for Sick Children) *Multi-factor ChIP-exo reveals TF binding dynamics within conserved orthologous cis regulatory modules in the mammalian liver*
16:00 – 17:00	Team Building Activity
18:30 - 20:00	Dinner (Fridolin-Simard)
Poster Session 2 (Salon Dupuis, Salle Esterel)	
20:00 - 22:00	See Poster Assignments (page 16)

Friday, October 7th, 2022

07:00 - 08:00	Breakfast *(Bistro à Champlain)*
Japan (AMED) - Canada (CIHR) International Collaborations Chair: Toshikazu Ushijima and Eric Marcotte	
08:00 - 08:30	**PROJECT: Single-cell probing of epigenetic memory in immunity** David Langlais (McGill University) *Epigenomics of innate immunity* Jay Shin (Genome Institute of Singapore A*STAR) *Functional characterization of cis-regulatory elements in health and disease*
08:30 - 09:00	**PROJECT: Artificial Intelligence based adaptive and interpretable models for analyzing multi-track epigenomic sequential data** Soichiro Yamanaka (Tokyo University) *Chromatin programming and reprogramming in mouse gonocyte* Ahmed Ashraf (University of Manitoba) *Artificial Intelligence based adaptive and interpretable models for analyzing multi-track epigenomic sequential data*
09:00 - 09:30	**PROJECT: Characterization of the integrative epigenetic and epitranscriptomic landscape of acute myeloid leukemias** Akihide Yoshimi (National Cancer Center Research Institute) *Crosstalk between Aberrant RNA Splicing and Epigenome Drives Tumorigenesis* Ly Vu (University of British Columbia) *The epitranscriptomic landscape of Acute Myeloid Leukemias*
09:30 – 10:00	Coffee Break
Epigenetic Programming Chair: Martin Hirst	
10:00 - 10:30	Will Pastor (McGill University) *ZMYM2 is essential for methylation of germline genes and active transposons in embryogenesis*
10:30 - 11:00	Guillaume Bourque (McGill University) *Transposable elements are a source of innovation and variability in our response to infection*
11:00 - 11:15	David Labbé (McGill University) *A targeted CRISPR/Cas9 screen identifies FOXA1 as a key transcription factor involved in MYC-driven and diet-dependent prostate cancer outcomes*
11:15 - 11:30	Sarah Grasedieck (University of British Columbia) *Sensitivity of myeloid leukemia cells to vitamin C is encoded in their regulatory DNA and chromatin topology*
CEEHRC Updates Moderator: Martin Hirst	
11:30 - 11:45	CEEHRC Network updates
11:45 - 12:00	**Close Meeting**
12:00	Bus departures

Poster Assignments

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5	Anene Nzelu, George	51	Hauduc, Axel	96	Padilla, Reinnier
7	Ayala Esparza, Jose Mauricio	52	Hawash, Mohamed	98	Pépin, Anne-Sophie
9	Azevedo Portilho, Nathalia	54	Hecker, Dennis	100	Porras, Lucas
11	Bentsen, Mette	56	Hossain, Ishtiaque	102	Radovani, Ernest
13	Bogutz, Aaron	58	Ialongo, Alyssa	104	Roemer, Anastasia
15	Bouzeraa, Lotfi	61	Katz, Megan	105	Rots, Dmitrijs
17	Brown, Joshua	63	Khader, Nawrah	107	Russett, Colleen
19	Chang, Kai-Wei	65	Kobayashi, Norio	108	Saini, Deepak
21	Chen, Xun	67	Kovac, Radmila	110	Sen, Shrinka
23	Cheng, Cheuk Yin	69	Kwon, Sin Young	112	Shipman, Gerry
26	Daneshpajouh, Habib	71	Lee, Kiera	114	Simon, Marie-Michelle
27	Darracq, Anaïs	73	Li, Qiao	116	song, mengyi
29	Doiron, Karine	75	Lussier, Alexandre	118	Taylor, Tiegh
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33	Duran Bishop, Gilberto	79	Malinowski, Alexandra	122	Villot, Romain
35	Elder, Elizabeth	82	Mathur, Vrinda	124	Wang, Li Qing
37	Espiritu, Daniel	83	Mehrjoo, Yosra	126	Wojtas, Bartosz
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6	Arcila Galvis, Juliana Estefania	49	Hamo, Dania	92	Ngubo, Mzwanele
8	Azarafshar, Pariya	50	Han, Jiyoung	94	Nie, David
10	Behjati, Fatemeh	53	He, Helen	95	Ondicova, Miroslava
12	Berthold, Anne	55	Horth, Cynthia	97	Paul, Racheal
14	Boufaied, Nadia	57	Hyacinthe, Jeffrey	99	Philipp, Lucas
16	Brewis, Hilary	59	Janssen, Sanne	101	Procaccini, Dena
18	Chan, Donovan	60	Johnston, Michael	103	Raynal, Noël
20	Chawla, Anjali	62	Keshavarzian, Tina	106	Rowland, Megan
22	Chen, Jocelyn	64	Kim, Jennifer	109	Sawchyn, Christina
24	Chik, Candus	66	Koborsy, Brenda Lynn	111	Shao, Xiaojian
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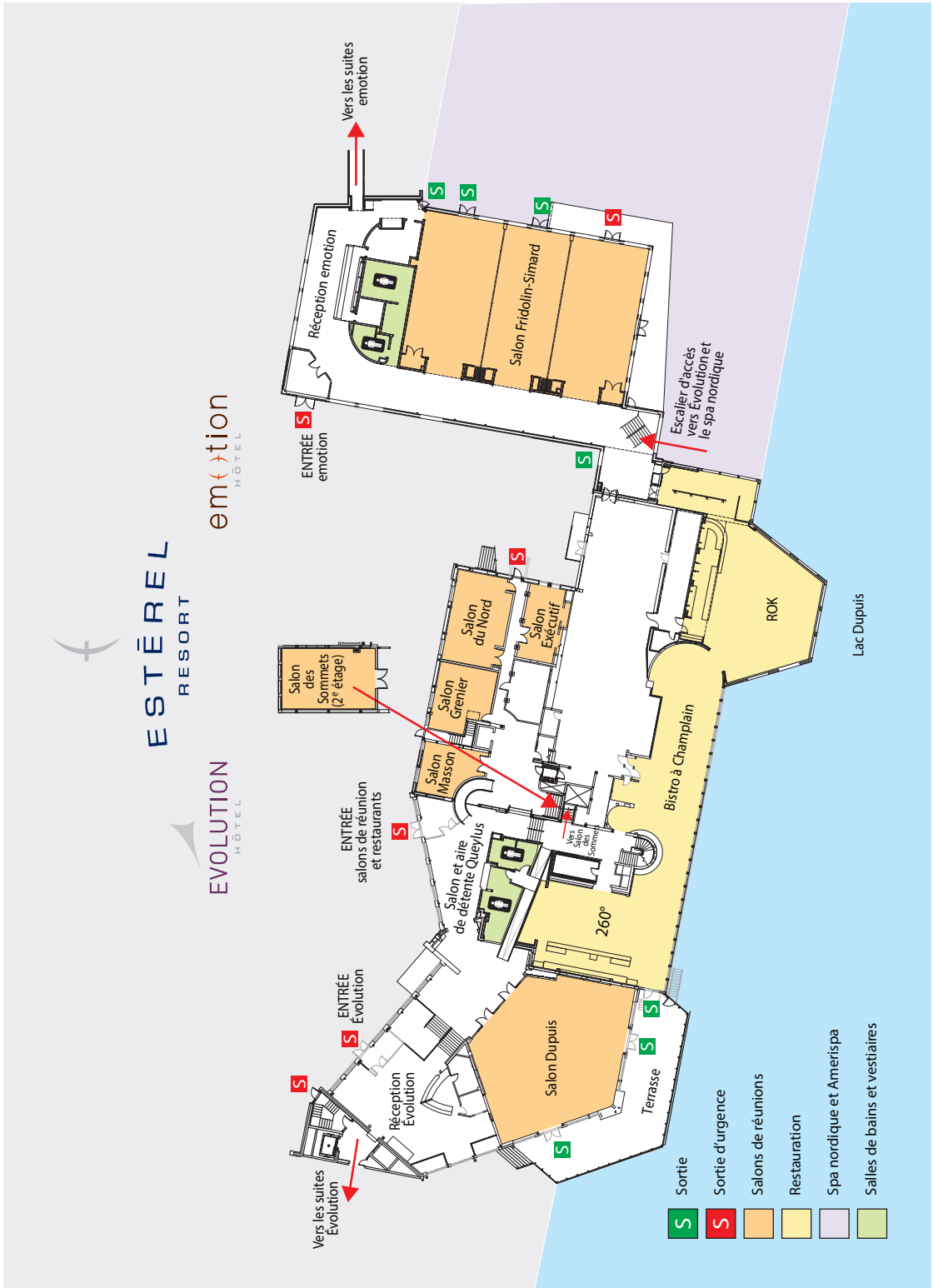

Abstracts

REGULATION OF THE BAP1 EPIGENETIC COMPLEX BY O-GLCNACYLATION

Louis Masclef[1], Oumaima Ahmed[1], Jessica Gagnon[1], Nicholas VG Iannantuono[1], Mila Gushel-Leclaire[1], Mohamed Echbicheb[1], Karine Boulet[1], Aurelio Balsalobre[2], Amandine Bemmo[2], Paul Lemire[1], Eric Bonneil[3], Frédérick A. Mallette[1], Pierre Thibault[3], Yoshiaki Tanaka[1], Jacques Drouin[3] and El Bachir Affar[1]
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We previously purified an epigenetic regulator complex that contain several chromatin regulators and notably the deubiquitinase and tumor suppressor BAP1. We established that BAP1 is a multiprotein complex that promotes transcriptional activation. BAP1 deubiquitinates histone H2AK119, a Polycomb group-mediated modification involved in gene silencing. BAP1 is frequently mutated in diverse cancers and represents the most frequently mutated deubiquitinase gene in the human genome. Our recent studies have revealed that important and dynamic post-translational modifications regulate several functions of the BAP1 complex. Interestingly, BAP1 interacts with the polycomb group protein OGT and the transcription factors FOXK1 and FOXK2. OGT is a unique enzyme that catalyzes the addition of an O-GlcNAc moiety to target proteins which impacts protein function including enzymatic activity and protein-protein interactions. This modification is also highly associated with fluctuations in cellular metabolism, as the donor substrate for the reaction, UDP-GlcNAc, is derived from the hexosamine synthesis pathway. Thus, OGT provides a link between cell environment and epigenetic regulation. Similarly, FOXK1 and FOXK2 have been shown to be implicated in cell proliferation, differentiation, and metabolic processes. We now found that FOXK1 but not FOXK2 is a novel substrate of OGT. Further, we found that this O-GlcNAcylation is modulated during the entry/exit of cell cycle. We also found that FOXK1 is critical for cell proliferation and that the interaction between FOXK1/BAP1 is compromised during nutrient starvation. Our studies revealed that OGT selectively modulates and regulates components of the BAP1 complex which orchestrates chromatin function and thus impact different cellular processes notably cell proliferation.

DNA METHYLATION MECHANISMS OF BDNF EXON IV AND VI IN PARTIAL BLADDER OBSTRUCTION, BEFORE AND AFTER DE-OBSTRUCTION

Tabina Ahmed[1,2], KJ Aitken[2], Martin Sidler[3], Sanaa Choufani[4], Priyank Yadav[5], Andrea Taylor[1,2], Suejean Park[1,2], Paul Delgado-Olguin[6], Rosanna Weksberg[7], DJ Bagli[6,8]
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Introduction: Partial bladder outlet obstruction (PBO) is a common urological condition that induces structural and functional bladder remodelling, and after removal of obstruction, its pathological features persist as a chronic obstruction bladder disease (COBD). The association between brain-derived neurotrophic factor (BDNF) and urinary complications have been studied in epidemiological and molecular contexts. Previous studies in our laboratory found that obstruction and decitabine treatment increased BDNF expression in smooth muscle and CpG methylation (CpGme) of BDNF, which correlated with function. In addition, COBD showed increases in BDNF variants 5 and 1. We speculate that specific epigenetic mechanisms – such as CpGme, hydroxymethylation (Hme) and histone3 lysine 27 trimethylation (H3K27me3) – may differentially alter expression of BDNF in both PBO and COBD. Thus, the purpose of this study is to compare epigenetic regulation of BDNF in COBD and PBO states, and to test the role of CpGme of BDNF. ***Methods:*** Archival tissue from PBO, COBD and sham rats were used. Briefly, obstructions were performed by ligating around the urethra plus a 0.9mm rod, which was then removed before closing the abdomen. COBD models were obstructions that were de-obstructed after 6 weeks and then followed for a further 4 weeks. Shams had sutures passed behind the urethra. DNA was isolated using Qiagen kits, and bisulfite-converted with EZ-DNA methylation gold kit (*Zymogen*). Pyrosequencing for DNA methylation was performed for the CGI, as well as for the shores and shelves of BDNF exons IV and VI in COBD. Chromatin isolation and ChIP were performed using the ChIP-iT Hi-Sensitivity kit for H3K27me3 IP, and the HmeDIP kit for Hme IP (both *Active Motif*). HmeDIP/PCR was performed in the untranslated (UTR) region, the upstream region, the 21kb enhancer region (which regulates multiple exons) and promoter region of Exon IV. dCas9 gRNA targeting the methylation of BDNF promoter is in progress using DNMT3A and TET catalytic domains and Puromycin selection. ***Results:*** COBD induced an increase in the BDNF exon IV isoform expression, which was reduced by decitabine treatment. PBO increased pan-BDNF, including exons IV and VI, but were conversely increased by decitabine treatment. While BDNF expression increased, the CpGme also increased at three sites in the promoter, coordinate with alterations in bladder function during obstruction. Bladder function (contractile efficiency) and collagen deposition were restored to normal with decitabine treatment during COBD. Hme of the exon IV 5' UTR were increased in both COBD and PBO (>0.02%) vs. sham (0.01%). Interestingly, H3K27me3 was significantly lost in chronic obstruction compared to sham, whereas PBO was not significantly altered. Upstream of exon IV 5' UTR, no changes were detectable. In the enhancer region 21 kb upstream, PBO showed 0.30+/-0.0028% Input in Hme. COBD had less Hme (0.21+/-0.0019% Input), though was still higher than sham (0.01+/-0.000093% input). ***Conclusions:*** The H3K27me3 and

Hme marks appear to combine and regulate BDNF, in both COBD and PBO. The increased Hme is consistent with the CpGme pyrosequencing (which does not differentiate between Hme and CpGme) and may thereby provide one underlying mechanism for CpGme changes and expression of BDNF during obstruction and COBD.

EPIGENETIC REGULATION OF A DOWNREGULATED GENE, KCNB2, IN OBSTRUCTIVE BLADDER DISEASE

K. J. Aitken[1], Martin Sidler[1,2], Priyank Yadav[1,3], Jia-Xin Jiang[1,4], Sanaa Choufani[5], Rosanna Weksberg[5,6] & Darius Bägli[1,4,7]
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Background: Partial bladder outlet obstruction is a widespread cause of urinary dysfunction and patient discomfort, resulting in immense health care costs. Even after ablation of the obstruction, functional and pathologic aspects of obstruction remain as a chronic obstructive bladder disease (COBD). Previously, we found that obstruction is associated with altered regulation of epigenetic machinery and altered function. Here we examined if COBD affects epigenetic marks in a proof of principle gene and explored mechanisms of its epigenetic regulation using *in vitro* models. **Methods**: COBD was created in 200-250g female Sprague-Dawley rats by surgical ligation of the urethra for 6 weeks, followed by removal of the suture and following animals for 6 more weeks. Obstruction is the 6-week ligation only. Sham ligations comprise passing the suture behind the urethra. Bladder function was non-invasively tested in metabolic cages at one day prior to de-obstruction and prior to sacrifice at 12 weeks. RT-PCR and pyrosequencing for DNA methylation (DNAme) was performed for KCNB2. To further understand the pyrosequencing results which do not differentiate between DNAme and DNA hydroxymethylation, chromatin IP/PCR for hydroxymethyl DNA (Hme) was assessed in several regions of the KCNB2 promoter. Another mark of epigenetic repression, histone3 lysine27 trimethylation (H3K27me3) was studied by ChIP/PCR. Rat bladder SMC were co-transfected by electroporation with puromycin or EGFP constructs of dCas9 fused with inactive or active catalytic domains of DNMT3A plus constructs of gRNA targeting promoter regions of *KCNB2*. Cells were plated onto damaged matrix (heat denatured collagen type I) to mimic the obstructive microenvironment and selected using Puromycin for days 1-7. DNA was extracted from cells for pyrosequencing. Parallel cells were immuno-stained for calponin and KCNB2 and analysed for EGFP co-localization of calponin and KCBN2 by Volocity 6.3. Cells on damaged matrix and native collagen were treated with vehicle or UNC1999, then analysed by immunostaining and western blotting for KCNB2, H3K27me3 and EZH2. ANOVA and *post hoc* t-test were performed, $p<0.05$=significant.

Results: RT-PCR revealed that KCNB2 expression was significantly downregulated during obstruction and COBD. Expression correlated downregulated DNA methylation and correlated with bladder function. However, both Hme and H3K27me3 increased during COBD in the CGI. 350 bp upstream of the CGI, fold enrichment of Hme increased during obstruction by >5 fold but not in COBD vs. sham. In contrast, the same regions had >20-fold enrichment of H3K27me3 during only COBD, but not obstruction vs. sham. In SMC *in vitro*, targeting specific DNA methylation sites using gRNA against the differentially methylated vs. other regions of the KCNB2 promoter increased DNA methylation in the targeted regions compared to other regions of the promoter. This targeting augmented KCNB2 expression and SMC differentiation. In SMC on damaged matrix, KCNB2 was inhibited and EZH2 increased. EZH2 inhibition restored KCNB2 expression and partially restored SMC phenotype.
Conclusions: Regulation of KCNB2 at the promoter demonstrated dynamic changes in DNA methylation, Hme and H3K27me3 during COBD and obstruction. *In vitro* models suggest that site specific DNAme upregulates and H3K27me3 downregulates KCNB2. Results of gRNA targeting with dcas-TET activity are in progress.

PARENT OF ORIGIN ASSIGNMENT OF HOMOLOGOUS CHROMOSOMES WITHOUT USING PARENTAL SEQUENCE DATA USING DNA METHYLATION SEQUENCING AND STRAND-SEQ

Vahid Akbari[1,2], Vincent C. T. Hanlon[3], Kieran O'Neill[1], Louis Lefebvre[2], Kasmintan A. Schrader[2,4], Peter M. Lansdorp[3], Steven J.M. Jones[1,2]
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While phasing algorithms are able to distinguish homologous chromosomes, parent-of-origin (PofO) assignment of phased homologs requires access to parental sequence data. Here we show that chromosome-scale phasing and PofO detection is possible without the need for parental sequencing information. We demonstrate that single-cell DNA template strand sequencing (Strand-seq) in combination with long-read Oxford nanopore sequencing allows construction of chromosome-scale haplotypes. Subsequently, we inferred DNA methylation at CpG dinucleotides from nanopore raw signals and used the known origin (Paternal or maternal) of methylation at human imprinted intervals to assign PofO to haplotyped homologs. We were able to assign PofO for autosomal chromosomes with an average mismatch error rate of 0.31% for SNVs and 1.89% for indels. Because our method can determine whether an inherited allele originated from the mother or the father, we predict that it will improve genealogy and the diagnosis and management of many genetic diseases where the phenotype is affected by the parental origin of the allele.

LONGITUDINAL MULTI-OMIC MONITORING OF PBMCS AT SINGLE CELL RESOLUTION IN CUTANEOUS MELANOMA PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS

Rached Alkallas[1,2,3], Yu Chang Wang[2], Spyridon Oikonomopoulos[2], Haig Djambazian[2], Ashot Harutyunyan[2], Eric Hongbo Xu[4], Nicholas R. Bertos[4], Jiannis Ragoussis[1,2,7], Catalin Mihalcioiu[4,5], Hamed S. Najafabadi[1,2], Ian R. Watson[3,4,6]
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The use of immune checkpoint inhibitors (ICIs) significantly improves the long-term outcome of metastatic melanoma patients. However, a significant number of patients do not respond to ICIs and many experience chronic immune-related adverse events. As immunotherapy using ICIs has now become part of the standard of care for melanoma patients, it will be critical to accurately predict who will benefit from this class of therapies. Currently approved biomarkers, including tumor mutation burden (TMB), mismatch repair (MMR) deficiency, and tumor PD-L1 expression offer modest predictive power. In addition to tumor-intrinsic factors, host-intrinsic factors such as the abundance of specific immune cells in the peripheral blood also have prognostic utility, as shown by recent studies. Importantly, recent pre-clinical studies have reported that exhausted CD8+ T cells, the key cellular targets of current ICIs, exist in a hierarchy of transcriptional and epigenetic states and that only specific subgroups within this hierarchy can be reactivated by ICIs. However, the scope and statistical power of these studies has been constrained by the use of a limited number of immune cell markers or limited number of human samples. To further explore the value of peripheral blood in predicting patient response to ICIs, we are performing multi-omic RNA and ATAC single-cell sequencing of peripheral blood mononuclear cell (PBMCs) procured before and during ICI therapy from a growing number of melanoma patients. In a pilot study including 16 patients, we jointly use epigenetic, transcriptional, and post-transcriptional information from single cells to identify cell types and cell states correlated with response to ICIs and integrate these biomarkers with matched tumor multi-omic data to develop a multivariate model of ICI response.

EPIGENETIC REPROGRAMMING LEADS TO METABOLIC CHANGES IN CHEMO-RESISTANT TRIPLE NEGATIVE BREAST CANCER

Gabriel Alzial[1,2], Tanya Bell[1], Charles Homsi[1,2], Aya Staili[1], Pierre Thibault[1,3], Geneviève Deblois[1, 2, 4]
[1]Institute for Research in Immunology and Cancer, Montreal, Canada; [2]Faculty of Medicine, University of Montreal, Montreal, Canada; [3]Department of Chemistry, Faculty of Arts and Sciences, University of Montreal, [4]Faculty of Pharmacy, University of Montreal, Montreal, Canada.

Triple-negative breast cancers (TNBC) comprise a heterogeneous subtype of breast tumors characterized by high incidence of chemotherapy resistance, resulting in poor outcome. Failure of drug response has been attributed to changes in tumor cell identity, which is largely influenced by epigenetic and metabolic reprogramming. Although these processes have previously been described in TNBC, their crosstalk and its consequences on TNBC progression and therapeutic responses are still poorly understood. Here, we hypothesize that epigenetic reprogramming can lead to changes in cellular metabolism that support chemo-resistance in TNBC. We observed significant changes in the global levels of specific histone acylation modifications in chemo-resistant TNBC cell models. We further show that these histone acylation changes are associated with the rewiring of TNBC cell metabolism, characterized by an increased dependency on glutamine and glutathione metabolism and enhanced mitochondrial respiration. We also identified the atypical chromatin localization of a mitochondrial metabolic enzyme regulating α-ketoglutarate availability in chemo-resistant cells, which may contribute to this epigenetic-metabolic rewiring. Importantly, while these adaptations give rise to both metabolic and epigenetic vulnerabilities that can be pharmacologically targeted to impair the survival of chemo-resistant TNBC models, we also show that targeting histone acylation can sensitize chemo-sensitive TNBC cells to pharmacological targeting of glutamine-related metabolic pathways. Together, our observations suggest an important rewiring of TNBC cellular metabolism in response to the dynamic remodeling of histone acylation marks that can be leveraged to reveal pharmacologically targetable vulnerabilities in TNBC.

SINGLE-CELL EPIGENOME ANALYSIS REVEALS AGE-ASSOCIATED DECAY OF HETEROCHROMATIN DOMAINS IN EXCITATORY NEURONS IN THE MOUSE BRAIN

Yanxiao Zhang[1*], Maria Luisa Amaral[2*], Chenxu Zhu[1], Steven F. Grieco[3], Xiaomeng Hou[4], Lin Lin[4], Justin Buchanan[4], Liqi Tong[3], Sebastian Preissl[4], Xiangmin Xu[3,5], Bing Ren[1,4,6,7#]
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Epigenomic alterations are an important and understudied aspect of aging. Although research into cell lines and bulk tissues have revealed some characteristics of aging at the chromatin level, including loss of heterochromatin, it is largely unclear what cell types and gene loci are affected. We address this knowledge gap by applying single cell epigenomic and multi-omic technologies to study aging. We probe the chromatin accessibility at single cell resolution in brain, heart, skeletal muscle and bone marrow from young, middle-aged and old mice, and assess age-associated changes at candidate regulatory elements (cCREs) across cell types and tissues. Unexpectedly, we detect increased chromatin accessibility within specific heterochromatin domains in

excitatory neurons in old mice. The gain of chromatin accessibility at these genomic loci is accompanied by a cell-type-specific loss of heterochromatin and activation of LINE1 elements. Immunostaining further confirms the loss of the heterochromatin mark H3K9me3 in these excitatory neurons but not in inhibitory neurons and glia cells. Our results reveal cell-type-specific changes in chromatin landscapes in old mice and shed light on the scope of heterochromatin loss in mammalian aging.

UNDERSTANDING THE ROLE OF CDK8 IN TRANSCRIPTIONAL REGULATION

Bhuvan Anbalagan[1], Zongchao Jia[2] and Maria Aristizabal[1]
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Transcription is the first step in the essential process of gene expression. In eukaryotes, RNA Polymerase II is the enzyme responsible for the transcription of all protein coding genes and several types of non-coding transcripts. The Mediator, transcription co-activator complex, is highly conserved across eukaryotic species and bridges information from transcription factors to RNAPII. Mediator consists of 25-30 subunits and is structurally divided into head, middle, tail and Cdk8 kinase module (CKM). CKM includes a subunit called Cdk8, a cyclin dependent kinase and the only catalytic subunit of the Mediator transcription co-activator complex. Functionally, Cdk8 activates or represses general or context-specific transcription by phosphorylating the carboxy terminal domain of RNA Polymerase II, several Mediator complex subunits, and a subset of transcription factors, many of which function in response to stress signals. Despite significant advances in our understanding of Cdk8 function, how Cdk8 is regulated is less clear. A recent high resolution crystal structure of the CKM by Li et al., showed that the N terminus of Med12(Med12N) interacts with the T-loop of Cdk8 which results in full activation of Cdk8 in vitro. To understand if the Cdk8-Med12 interaction regulates Cdk8 activity in vivo, I generated Saccharomyces cerevisiae mutants carrying Cdk8 or Med12 alleles that strengthen and weaken the Cdk8-Med12 physical interaction and examined their effects on growth and CDK8-dependent gene expression. My results so far indicated that the Cdk8-Med12 interaction is important for Cdk8 activity in vivo and may be regulated in a context-specific manner. More specifically, I observed opposing growth effects for strains carrying alleles that strengthen Cdk8-Med12 and strains carrying alleles that weaken the interaction. Furthermore, in some conditions, it was the strain carrying the Cdk8-Med12 interaction strengthening allele that showed wild-type growth, while it was the strain carrying the Cdk8-Med12 interaction weakening allele that showed wild type growth in other conditions. These results provide an important starting point to understand how Cdk8 is regulated in vivo, an important question given an emerging role of CDK8 as an oncogene in colorectal cancer and other types of cell malignancies.

CTCF MEDIATES THE ACTIVITY-BY-CONTACT DERIVED CIS-REGULATORY HUBS

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The 3D chromatin architecture establishes a complex network of genes and regulatory elements necessary for transcriptomic regulation in development and disease. This network can be modeled by cis-regulatory hubs (CRH) which underscore the local functional interactions between enhancers and promoter regions and differ from other higher order chromatin structures such as topological associated domains (TAD). The Activity-by-contact (ABC) model of enhancer-promoter regulation has been recently used in the identification of these CRHs, but little is known about the role of CTCF on the ABC scores and the consequent impact on CRHs.
Here we show that the loss of CTCF leads to a reorganization of the enhancer-promoter interactions resulting in a re-distribution of ABC scores of the putative enhancers. The loss of CTCF also leads to a global reduction of the total number of CRHs, and an increase in the size of the CRHs due to increase in the number of elements within each hub. In addition, CTCF loss led to more CRHs that cross TAD boundaries.
These results provide another layer of evidence to support the importance of CTCF in the formation of regulatory networks necessary for gene regulation. Thus, mutations that affect genomic binding of CTCF could impact overall strength of enhancers and dysregulation of genes within a hub.

CHARACTERIZATION OF THE MONOCYTE LINEAGE ENHANCERS AND THEIR ROLE IN DISEASE

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Monocytes and their descendant cell types play an important role in the chronic inflammation associated with many complex immune-mediated diseases. Genome-wide association studies (GWAS) have identified that most of the genetic variants associated with complex diseases are in non-coding regions. Enhancers are non-coding sequences that dynamically regulate the expression of distal target genes via chromatin looping and binding of transcription factors. Changes in the DNA of enhancers

can alter their affinity for transcription factors and other DNA-binding proteins, which can contribute to phenotype changes and disease.
Using ChiPSeq data from the BLUEPRINT Consortium, we produced a whole-genome annotation of chromatin states denoting heterochromatin, elongating genes, repressed, and active regulatory regions for 108 samples spanning 31 cell types of the human hematopoietic lineage. We characterized in depth the enhancers of primary monocytes and six descendant cell types differentiated *in vitro*: osteoclasts, macrophages (M0, M1 and M2), immature, and mature dendritic cells.
We found that poised enhancers are about twice more frequent than active enhancers (81.5Mb vs 41.4Mb, on average). In comparison to monocytes, descendant cell types tend to gain more enhancers (32.8Mb active, 65.3Mb poised) than they lose (6.1Mb active, 21.6Mb poised). We dissected the enhancer regions based on their activity across cell types, we tested for their enrichment in non-coding variants associated with GWAS diseases and traits. We found variants associated with osteoarthritis and cardiovascular disease in inflammatory M1 macrophages; autoimmune diseases, arthritis and calcium levels enriched in enhancers of dendritic cells; while in osteoclasts we found enrichment of bone phenotypes. Our next aim is to identify the genes regulated by the macrophage enhancers enriched in disease-associated variants, using promoter-capture HiC.

GLUTATHIONE METABOLISM IS A REGULATOR OF THE ACUTE INFLAMMATORY RESPONSE OF MONOCYTES TO (1→3)-B-D-GLUCAN

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Invasive fungal infections are increasingly becoming a global health issue causing more than 150 million severe or life-threatening cases each year worldwide. With the drastic increase of the at-risk population, including the elderly, immunocompromised and critically ill, effective therapies are crucial to counteract the mortality and morbidity of these infectious agents. (1→3)-β-D-Glucan (BDG) represents a potent pathogen-associated molecular pattern (PAMP), capable of triggering the host response to fungal and certain bacterial infections. Members of our innate immune system, specifically circulating monocytes, are known to serve an integral role in recognizing PAMPs such as BDG and initiating a potent immune response by phagocytosing/presenting antigens, as well as alarming and recruiting other members of the immune system to the site of infection. Yet, while the acute response by monocytes is widely recognized as a critical component of the host's response to various infectious agents, the mechanistic pathways involved and how they interact in said response remain to be fully understood. Exploring previously published epigenetic and transcriptomic data, as well as well via *in vitro* experimentation, we investigated the pathways involved in the host response to BDG by primary human monocytes, at the epigenetic, transcriptomic, and molecular levels. To this end, we analyzed RNA-seq, ATAC-seq, H3K27ac and H3K4me1 ChIP-seq data from human monocytes at 1, 4, and 24 hours post BDG exposure. Gene enrichment analysis demonstrated that multiple metabolic pathways such as glutathione metabolism, pentose phosphate pathway citric acid cycle (PPP) and cholesterol metabolism were upregulated by monocytes in response to BDG exposure. Interestingly, in contrast to lipopolysaccharides, which increased the intracellular GSH/GSSG ratio without changing total glutathione levels, BDG increased both the GSH/GSSG ratio and the total amount of intracellular GSH. Moreover, BDG induced NADP synthesis, increased NADPH/NADP ratio, and increased expression of genes involved in the pentose phosphate pathway in a GSH-dependent manner. Furthermore, by inhibiting GSH synthesis with L-buthionine sulfoximine (BSO) before BDG exposure, we show that GSH is required for monocyte survival and surprisingly is an important regulator of BDG-induced nitric oxide secretion, phagocytosis, and cytokine production. In summary, our work expands our knowledge of the host immune response by revealing that BDG induces glutathione synthesis and metabolism, an important regulator of the acute functional response of human monocytes.

CHARACTERIZING THE ROLE OF HISTONE H3.3 K36E GERMLINE POINT MUTATION IN NEURODEVELOPMENT

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H3.3, the non-canonical variant of Histone 3, is subject to a variety of post-translational modifications on its N—terminal tail. Somatic mutations in H3.3 encoding genes (*H3F3A* and *H3F3B*), which result in amino acid substitutions on the H3.3 tail, are responsible for different types of malignancies. However, germline mutations in H3.3 encoding genes correlate with neurodevelopmental disorders in human patients. Recently, a male patient suffering from developmental delays, seizures, and craniofacial abnormalities with the *H3F3A* K36E germline point mutation has been reported. H3K36me3 is correlated with gene activation which can be tri-methylated by the activity of SETD2 methyltransferase and can be recognized by ZMYND11 reader.
We hypothesize that the H3K36E substitution may disrupt the activity of SETD2 *in-cis* and potentially disrupt ZMYND11 protein binding to H3K36**.** We generated a Direct-Knock-In (DKI) heterozygous *H3f3a* K36E mouse model to study this mutation and investigate the effects of the mutation on mouse development, neurodevelopment, and behavior. Interestingly, we observed that our mouse model developed abnormal behaviors, including aggression, hyperactivity, anxiety, self-mutilation, and in general ASD-like behaviors. Next, we assessed the distribution of various brain populations (neurons, astrocytes, and oligodendrocytes), but surprisingly, no overt differences were detected. Furthermore, by using epigenomic, genomic, and

transcriptomic tools such as Next-Generation-Sequencing (NGS), we will analyze the effects of this mutation on post-translational modifications, genome, and gene expression pattern, respectively. This study will give further insights into the role of H3K36 residue and H3K36E substitution in chromatin remodeling, development, and specifically brain neurodevelopment.

A NON-COVALENT DNMT1 INHIBITOR MEDIATES GLOBAL DEMETHYLATION IN MURINE EMBRYONIC STEM CELL

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Background: DNA methylation plays an important role in regulating gene expression in mammals. The covalent DNMT1 inhibitors 5-azacytidine and decitabine are widely available for used in research to reduce DNA methylation levels, but they impart severe cytotoxicity which limits their demethylation capability and confounds interpretation of experiments. Recently, a non-covalent inhibitor of DNMT1 called GSK-3484862 was developed by GlaxoSmithKline. We sought to determine whether GSK-3484862 can induce demethylation more effectively than 5-azanucleosides. Murine embryonic stem cells (mESCs) are an ideal cell type in which to conduct such experiments, as they have a high degree of DNA methylation but tolerate dramatic methylation loss. Results: We determined the cytotoxicity and optimal concentration of GSK-3484862 by treating wild- type (WT) or Dnmt1/3a/3b triple knockout (TKO) mESC with different concentrations of the compound, which was obtained from two commercial sources. Concentrations of 10 μM or below were readily tolerated for 14 days of culture. Known DNA methylation targets such as germline genes and GLN-family transposons were upregulated within two days of the start of GSK-3484862 treatment. By contrast, 5-azacytidine and decitabine induced weaker upregulation of methylated genes and extensive cell death. Whole genome bisulfite sequencing (WGBS) showed that treatment with GSK-3484862 induced dramatic DNA methylation loss, with global CpG methylation levels falling from near 70% in WT mESC to less than 18% after six days of treatment with GSK-3484862, similar to the methylation level observed in Dnmt1 deficient mESCs. Conclusions: GSK-3484862 mediates striking demethylation in mESCs with minimal non-specific toxicity.

GAZE: A SINGLE-CELL GENE REGULATORY INFERENCE FRAMEWORK FOR INTEGRATING TRANSCRIPTOMIC AND EPIGENOMIC DATA

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Single-cell sequencing has become a prevalent approach to interrogate cell-type specific signatures and cellular heterogeneity, which assists the researchers to unravel the underlying complexities of diseases. This, however, creates a need for integrating single-cell omics data by specialized machine learning approaches that are capable of inferring key regulatory players at single-cell granularity. Although there have been numerous methods proposed for discovering transcriptional regulation on the basis of scRNA-seq data, they lack delivering a comprehensive view of the whole regulatory landscape.
Here, we address these limitations by incorporating diverse single-cell modalities. Through employing a multi-task learning approach, we have established a versatile statistical framework, called GAZE, that guarantees a comprehensive analysis of single-cell data in an integrative fashion. Using transcription factors (TFs) as the main regulators, we infer associations between TFs and genes regulating any single cell given.
This allows us to broaden the current understanding of transcriptional regulatory mechanisms through identifying the key players involved in differential regulation of various cell types.
Additionally, we designed suitable tests to investigate the biological relevance of the inferred regulatory activities, and prioritize genes or TFs, that drive cell differentiation and explain observed heterogeneity.
We currently have examined the results on various scRNA-seq samples of patients suffering from heart failure. We illustrate our results with visualizations of our Shiny application implemented in R. The suggested framework is general and allows extension to other types of regulators, such as miRNAs, which will be explored in the future.

BIOINFORMATICS ANALYSIS OF HIGH-THROUGHPUT EPIGENETICS ASSAYS TO UNCOVER TRANSCRIPTION FACTOR ACTIVITY AND CO-OCCURRENCE NETWORKS

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Epigenetic regulation of gene expression is crucial for all processes occurring in cell biology, and in this context, transcription factors (TFs) play important roles in targeting particular genes. They are expressed at particular time points during e.g. differentiation to drive transcriptional programs, either acting individually or deploying activity in larger TF complexes. However, while recent advancements of high-throughput sequencing methods generate massive amounts of data on epigenetics and TF binding, there is an increasing need for robust bioinformatics tools to study, interpret and integrate the data. Thus, our work centers on expanding the bioinformatics analysis of existing data to uncover the interplay and regulation of TF binding networks.
In order to identify potential TF binding sites in rare cell types, we developed TOBIAS (Transcription factor Occupancy prediction By Investigation of ATAC-seq Signal; *Bentsen et al., NatComm, 2020*), which utilizes TF footprinting to predict TF binding. Based on ATAC-seq or SC-ATAC-seq data, TOBIAS finds genomic positions where chromatin is protected by Tn5 cutting due to binding of TFs - positions known as footprints. These footprints are then correlated with known TF sequence preference in a

genome-wide manner. From TOBIAS analyses in multiple cell types and organisms, it became apparent that distinct TFs often act together in the same biological conditions - a concept known as *TF co-occurrence*. In order to detect co-occurring TF binding sites, we developed the TF-COMB (Transcription Factor Co-Occurrence using Market Basket analysis) framework, which utilizes a market basket analysis (MBA). MBA has classically been applied to investigate shopping habits such as "if the customer buys cereal, they are likely to buy milk", however, this approach can be applied to TF co-occurrence analysis like "if TF1 binds, it is also likely that TF2 binds" as well. Our co-occurrence analysis on TF ChIP-seq and/or ATAC derived footprinting locations uncovers an organized network of TF-TF co-occurrences, which also exhibits a particular syntax in terms of distance, binding site orientation and relative location in open chromatin. Interestingly, this observation of *binding grammar* also extends to the co-occurrence of TFs with known histone modifications and genomic elements, as we uncovered groupings of TFs specifically co-localizing within enhancers, promoters and even closed chromatin.
In conclusion, our toolbox significantly extends standard analysis on ATAC/ChIP data and thereby helps to unravel the influence of TF binding within a diverse set of biological conditions. It is open to a multitude of omics data formats and comes with robust pipelines making them applicable to a variety of cells, tissues and organisms.

RNA SEQUENCING OF HUMAN CELLS PROVIDES INSIGHT INTO THE ROLE OF NON-CODING RNAS AND GENES AFFECTED DURING INFECTION WITH THE LYME DISEASE PATHOGEN BORRELIA BURGDORFERI

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Borrelia burgdorferi is the etiological agent causing Lyme disease, which is affecting an increasing number of people worldwide. The interaction of tick-borne Borrelia with the host in the early phase of infection is not yet fully understood. Changes in epigenetic mechanisms, such as the regulatory effect of non-coding RNAs, triggered by Borrelia have not yet been sufficiently explored. We hypothesize that bacteria interfere with the host epigenetics and thus influence the activity of genes. Therefore, the main objective of this study is to identify genes and micro-RNAs significantly affected by the presence of Borrelia in human cell models. Two cell models (HUVEC and HEK-293) were exposed to Borrelia burgdorferi for 72 h, a time consistent with tick feeding and infection establishment in the host. Total RNA extracts were subjected to whole-genome RNA-seq and small RNA-seq based on size selection using a Novaseq 6000 (Illumina). Standard bioinformatics pipelines were used to identify significant genes (DeSeq2) and microRNAs (Compsra). Data integration was performed with multimir analysis and visualized in Cytoscape. We found more differentially expressed genes (adjusted p-value<0.05) with a stronger fold change in HUVEC (364 in total) in comparison to HEK-293 (110 in total). Enriched Gene Ontology terms of Biological Processes such as INF-response and anti-viral response were identified in HUVEC. For HEK-293 the genes were mainly enriched in functional terms like wound healing and extracellular matrix organization, in which the majority of genes were downregulated. 78 differentially expressed miRNAs (adjusted p-value<0.05) were identified for HUVEC, whereas in HEK-293 75 differentially expressed miRNA (adjusted p-value<0.05) were found. The identified microRNAs were both up- or downregulated. The association of DEGs with the microRNAs reinforces functions with significant genes. Overall, our results suggest that non-coding RNAs are involved in cellular signaling in humans in the response to Borrelia infection, opening an exciting new avenue to explore the molecular and epigenetic mechanisms involved in Lyme disease.

BET FAMILY INHIBITION FOR PULMONARY ARTERIAL HYPERTENSION: THE APPROACH TRIAL

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Background: Bromodomain-containing protein 4 (BRD4), a member of the Bromodomain and Extra-Terminal motif family, has been identified as a critical epigenetic driver for pulmonary arterial hypertension (PAH). Apabetalone, a clinically available BRD4 antagonist, was shown to reverse pulmonary artery remodeling in diverse PAH rat models, as well as to support the pressure-loaded right ventricle.
Objectives: The aim of this pilot study was to assess the feasibility of a future early-stage clinical trial evaluating BRD4 inhibition in PAH and to provide preliminary evidence that apabetalone may be effective in PAH.
Methods: This was a two-centre, open-label, single-arm, 16-week study evaluating apabetalone 100mg BID in PAH (NCT03655704). Eligibility criteria included idiopathic PAH (IPAH) or PAH associated with connective tissue disease (PAH-CTD) adult patients (18-75 yrs) with pulmonary vascular resistance (PVR)>480 dyn.s.cm^{-5} and a six-minute walk test (6MWT)>150m despite stable guideline-recommended therapy. Baseline and post-baseline tests included a right heart catheterization, and serial 6MWT and NT-proBNP assessments. In addition to feasibility, exploratory efficacy endpoints included changes in PVR (predefined key efficacy endpoint), as well as changes in other pulmonary hemodynamic parameters, 6MWT and NT-proBNP. Changes in exploratory efficacy endpoints are reported on a descriptive basis with 95% confidence intervals.
Results: Amongst the 9 subjects screened from 09/2019 to 05/2021, 2 were excluded due to baseline PVR <480 dyn.s.cm^{-5}. The 5 IPAH and 2 PAH-CTD enrolled participants (5 females, mean age 56±8yo, 5 and 2 on dual and triple therapy, respectively) completed the study procedures without dose reduction or discontinuation. Two patients experienced an asymptomatic 1.5 and 2.4 times the upper limit of normal increase in transaminases that spontaneously resolved despite continued treatment. No serious adverse events were reported.

Conclusions: Consistent with previous studies in cardiovascular diseases, apabetalone was well tolerated and all patients completed study-related procedures. Exploratory analysis also suggested potential improvements in PVR and cardiac function while gaining some experience on tolerability. These results should obviously be interpreted with caution given the exploratory nature of these analyses and the open-label design of the study. A multicenter, randomized, double-blind, placebo-controlled trial (NCT04915300) to assess the efficacy of apabetalone for the treatment of PAH compared to placebo in currently ongoing.

CHIP-SEQ SPIKE-IN NORMALIZATION: THE GOOD, THE BAD, AND THE UGLY

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Chromatin Immunoprecipitation followed by Sequencing (ChIP-seq) has opened a window on the epigenetic landscape of cells previously viewed through the pinhole of ChIP-qPCR. ChIP-seq allows genome-wide looks at chromatin signatures in whatever cell type and genetic background we can derive. Visualization and quantitation of ChIP-seq signal involves normalization steps to allow for cross-sample comparisons, but this normalization has unintended consequences. One of the most widely used normalization is Counts Per Million (CPM), in which the number of sequenced reads over a region of interest is divided by the total number of reads found in the sample, then multiplied by a million. This allows samples with varying depths of sequencing to be directly compared, and the numbers produced are often intuitive for researchers. Most normalizations (RPM, RPKM, FPKM) incorporate a similar metric, with total read count in the denominator. In effect, though, this scales signal across the entire genome, where the sum of signal should equal one million for each sample. Some cell types and mutant backgrounds, though, will result in varying absolute quantities of signal, and these differences will be lost in this standard normalization step. To ameliorate this problem, several flavours of Spike-In material have been proposed for ChIP-seq experiments. Here I will evaluate some options available for researchers and make recommendations for choice of Spike-In material as well as downstream data processing steps.

MYC DRIVES AGGRESSIVE PROSTATE CANCER BY DISRUPTING TRANSCRIPTIONAL PAUSE RELEASE AT ANDROGEN RECEPTOR TARGETS

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c-MYC (MYC) is a major driver of prostate cancer tumorigenesis and progression. Although MYC is overexpressed in both early and metastatic disease and associated with poor survival, its impact on prostate transcriptional reprogramming remains elusive. We demonstrate that MYC overexpression significantly diminishes the androgen receptor (AR) transcriptional program (the set of genes directly targeted by the AR protein) in luminal prostate cells without altering AR expression. Analyses of clinical specimens reveal that concurrent low AR and high MYC transcriptional programs accelerate prostate cancer progression toward a metastatic, castration-resistant disease. Data integration of single-cell transcriptomics together with ChIP-seq uncover an increase in RNA polymerase II (Pol II) promoter-proximal pausing at AR-dependent genes following MYC overexpression without an accompanying deactivation of AR-bound enhancers. Our study revealed an intricate crosstalk between the AR, MYC, FOXA1 and RNA Pol II resulting in a corrupted AR transcriptional program and promoting prostate cancer initiation and progression to the metastatic castration-resistant prostate cancer stage. Altogether, our findings suggest that MYC overexpression antagonizes the canonical AR transcriptional program and contributes to prostate tumor initiation and progression by disrupting transcriptional pause release at AR-regulated genes. Considering that a simple dietary intervention meant to reduce saturated fat consumption can dampen MYC transcriptional program, and the recent development of viable MYC inhibitors for therapeutic interventions, we foresee that targeting MYC may help restore a canonical AR transcriptional program and sensitize prostate cancer to AR-targeted therapies.

CONTRIBUTION OF THE METHYLOME IN THE IDENTITY AND FUNCTIONING OF IMMUNE CELLS IN BOVINES

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The sustainable livestock sector is subject to various intrinsic factors (physiological stages, life course) and extrinsic factors (nutrition, livestock management, heat stress, etc.), which point to the necessity for a better characterization of adaptive capacities, and in particular immune skills of the animal. Hence, it was appeared the concept of the "robust animal" that is able to adapt to environmental changes and to remain in good health. The exploitation of the genetic heritage and the development of genomic selection are already widely used to select animals on traits of interest and to offer recommendations for breeding management considering the breed diversity and individual variabilities of immune response. However, the immune skills of the individual remain poorly inheritable (h^2<10-15%) and based on the establishment and interaction of multiple subpopulations of immune cells with specific functions. Immune cells are derived from hematopoietic stem cells (HSCs). HSCs differentiate into lymphoid and myeloid progenitors that further branch out to the more specific cell types associated with adaptive and innate immunity throughout the life of the individual.
In addition, of the transcriptional control mechanisms involving transcription factor networks, epigenetic processes, including DNA methylation and post-translational modifications of histones provide a molecular basis for cellular memory and cell identity maintenance. The aim of this study was identification of DNA methylation differences apposed on bovine genome and involved in the identity of immune cells (lymphocytes (CD4), monocytes (M) and neutrophils (N)) and to determine the variability between individuals during the career. Tracking these marks will provide precision phenotyping of immune competence and offer a new approach to identifying the most robust animals.
For 36 Prim'Holstein cows at the same physiological stage and free from disease, pan-genomic data were analyzed for the DNA methylation by RRBS "Reduced Representation Bisulfite Sequencing" using genomic DNA from different purified sub-populations of immune cells (CD4, M, N). On 1.3 million $CpG_{10\text{-}500}$, the differential analyzes (Methylkit), carried out two by two (with a Δ of methylation > 25%) make it possible to establish a list of DMC (Differentially Methylated Cytosine) for each comparison (DMC of MvsN, CD4vsN and CD4vsM). Thus, 63505 DMCs are identified among which 45439 signatures are specific for CD4, 3874 signatures - specific for N and 3899 - specific for M; these respective numbers of signatures being consistent with the initial differentiation between lymphoid and myeloid progenitors, then between polynuclear and mononuclear cells. In 80% of cases, these signatures targeted a gene or its putative regulatory regions (10 kb upstream and downstream of the gene). The identification of the genes targeted by the DMCs and the enrichment analysis of the networks and/or signaling pathways suggested different functions for the three cell subpopulations: the $DMC_{Monocytes\ and\ neutrophils}$ (Monocytes – 712 genes and Neutrophils – 768 genes) target genes mainly involved in cell function (*pvalue* from $1.3x10^{-6}$ to $2.8x10^{-4}$). $DMC_{lymphocytes}$ (4638 genes) are mainly associated with the control of the transcriptional machinery (*pvalue* from $1.8x10^{-22}$ to $2.3x10^{-14}$). These data allow us to establish working hypotheses regarding the epigenetic control of the immune response in bovine species.

WHAT MAKES A HISTONE VARIANT A VARIANT: CHANGING H2A TO BECOME H2A.Z

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Chromatin structure and underlying DNA accessibility is modulated by the incorporation of histone variants. H2A.Z, a variant of the H2A histone family, plays a distinct and essential role in a diverse set of biological functions including gene regulation and maintenance of heterochromatin-euchromatin boundaries. Although it is currently unclear how the replacement of H2A with H2A.Z can regulate gene expression, the variance in their amino acid sequence likely contributes to their functional differences. To tease apart regions of H2A.Z that confer its unique identity, a set of plasmids expressing H2A-H2A.Z hybrids from the native H2A.Z promoter were examined for their ability to recapitulate H2A.Z function. First, we found that the H2A.Z M6 region was necessary and sufficient for interaction with the SWR1-C chromatin remodeler. Remarkably, the combination of only 9 amino acid changes, the H2A.Z M6 region, K79 and L81 (two amino acids in the α2-helix), were sufficient to fully rescue growth phenotypes of the *htz1Δ* mutant. Furthermore, combining three unique H2A.Z regions (K79 and L81, M6, C-terminal tail) was sufficient for expression of H2A.Z-dependent heterochromatin-proximal genes and *GAL1* derepression. Surprisingly, hybrid constructs that restored the transcription of H2A.Z-dependent genes, did not fully recapitulate H2A.Z-specific enrichment in chromatin. This suggested that H2A.Z function in transcription regulation may be at least partially independent of its specific localization at gene promoters. Together, this research has identified three regions that can confer specific H2A.Z-identity to replicative H2A, furthering our understanding of what makes a histone variant a variant.

HISTONE ACETYLATION AND TRANSCRIPTION RESTART IN BUDDING YEAST

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Histone acetylation marks are often associated with gene expression and may depend in part on active transcription. However, it is not fully understood whether this acetylation contributes to ongoing transcription. We sought to reversibly inhibit transcription with 1,10-phenanthroline (1,10-PT) in order to determine if acetylation marks established at transcribed genes are important for future transcription. We first examined the effects of 1,10-PT, and found that the general transcription factor TFIIB was depleted from specific gene promoters after 1,10-PT. This was similar to recent results which suggest that transcription inhibition can deplete general transcription factors from gene promoters. To examine restart from this transcription inhibition, we washed away 1,10-PT from treated cells, which rapidly

restored transcription. We will examine this restart of transcription in mutants lacking histone modification sites and determine if any marks promote transcriptional restart. If any mutants show a delay in transcription restart, we also examine their impact during recovery from other transcription inhibitors.

LINKING ALTERED RETROTRANSPOSON DNA METHYLATION IN MALE GERM CELLS WITH INTERGENERATIONAL INHERITANCE THROUGH THE PATERNAL LINE.

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There have been numerous examples of different parental germline exposures resulting in adverse health outcomes in future generations. Epigenetic inheritance has emerged as a mechanism mediating intergenerational effects.
We and others have shown that epigenetic marks, such as DNA methylation, modifications to histones or sncRNAs, can be altered due to environmental exposures. These marks can potentially provide a means for intergenerational inheritance. While global epigenetic reprogramming occurs, removing the majority of these marks post-fertilization, some sequences are resistant to erasure, thereby allowing the possibility for generational epigenetic inheritance.
Repetitive elements, such as retrotransposons, are sequences that are able to escape epigenetic reprogramming that occurs postfertilization and in primordial germ cells. While they were first viewed as "junk" DNA, studies have now demonstrated their importance in generating genetic diversity, response to environmental cues and help in regulating gene expression. Indeed, they play an important role in embryogenesis, as they are activated and highly expressed in preimplantation embryos.
We have used several models to study DNA methylation in male germ cells and the effects of various exposures and gene defects on subsequent generations: 1) folic acid supplementation and deficiency, 2) knock-outs of 5,10-methylenetetrahydrofolate reductase (MTHFR) and 3) DNA methyltransferase 3-like (DNMT3L) haploinsufficiency. Both folic acid and MTHFR are important components of the one-carbon metabolism pathway, which supplies methyl groups that are eventually used in methylation reactions, such as those modifying DNA and histone. While DNMT3L does not process catalytic DNA methyltransferase activity, it plays a vital role in germ cell development, as complete lack of this enzyme results in male sterility.
In these three different models, we have examined DNA methylation in mature spermatozoa and found dramatic losses of DNA methylation, particularly in intergenic regions of the genome. Interestingly, these hypomethylated regions are enriched for retrotransposons such as LINEs and LTRs; particularly young LINE-1 elements were found to be over-represented compared to background. As well, in these models, we have observed abnormal phenotypes in the subsequent generations: folic acid supplementation/deficiency resulted in decreased litter sizes; *Mthfr*$^{-/-}$ led to a worsening of reproductive phenotypes across generations; while sperm from *DNMTL3*$^{+/-}$ mice, when combined with the use of assisted reproductive technologies, resulted in increased numbers of embryos with birth defects.
Based on evidence from the three models, we hypothesize that retrotransposons, and in particular young, potentially active retrotransposons, that enter embryogenesis in a hypomethylated state, may play an important role in abnormal development and epigenetic inheritance. Aberrant expression of these retrotransposons in preimplantation embryos could contribute to birth defects later in gestation. To lead to abnormalities in subsequent generations, we propose that the hypomethylated retrotransposons partially escape DNA remethylation that occurs at the time of implantation. To test this, we are examining whether increased expression of retrotransposons can be observed in both germ cells and embryos through several techniques including RNA-seq, qRT-PCR and immunohistochemistry.

DISSECTING THE ROLES OF LTR ELEMENTS IN GENOMIC IMPRINTING

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Genomic imprinting distinguishes the developmental roles of maternal and paternal genomes. In mammals, stable genomic imprinting is achieved via parent-of-origin DNA methylation (DNAme), an epigenetic marking heavily involved in regulating gene expressions. During germ cell development, germline cells experience erasure of epigenetic marks followed by *de novo* establishment of sex-specific DNAme to form mature gametes. During pre-implantation, a wave of demethylation occurs, in which few gametic differentially methylated regions (gDMRs) are protected via DNAme maintenance mechanism: these are the imprinted gDMRs (igDMRs). We previously reported that long-terminal-repeat retrotransposons (LTRs) transcriptionally active in oocytes are responsible for the wide differences in DNAme patterns in different species. Additionally, such transcription coupled *de novo* DNAme in fully grown oocytes (FGOs) are highly associated with the initiation of maternal imprinting. Our previous study on imprinted genes *Slc38A4* and *Impact* showed their respective upstream LTRs on murine genome act as alternative promoter in FGO. This leads to the acquisition of DNAme at their canonical promoter and subsequent imprinting in somatic cells. Given that LTRs are remnants of ancient retroviral invasion, the insertion of LTR may likely introduce transcription-coupled *de novo* DNAme that could lead to imprinting should it provide evolutionary advantages. To highlight the evolutionary potential of this hypothesis, we are conducting gain-of-function studies by introducing oocyte-active LTR to the upstream of a non-imprinted gene in mouse. *Zc3h12c* is the murine non-imprinted and human imprinted gene we selected for the CRISPR-Cas9 mediated knock-in of the oocyte-active MTA LTR element from the *Bmp5* locus. We have successfully generated knock-in mouse line ready to be analyzed for transcription-coupled *de novo* DNAme and imprinting signatures. Our experimental design will also investigate the imprinting initiation and maintenance depend on the presence of the LTR elements. We anticipate the outcomes will provide comprehensive evidence to the role of repeat

elements in imprinting through transcription-coupled DNAme deposition.

SEX- AND CELL-TYPE-SPECIFIC GENE REGULATORY CHANGES IN MAJOR DEPRESSIVE DISORDER

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Research Problem: Major Depressive Disorder (MDD) is the leading cause of lifelong disability and a major risk factor for suicide. The sex-specific impacts of depression are not fully understood. Genome-wide association studies (GWAS)-identified genetic variants for most psychiatric disorders are disproportionally located in the non-coding regions of the genome, whose functions are unknown [1]. In addition, GWAS meta-analyses have found that MDD-associated genetic risk variants are largely enriched in the prefrontal cortical cell-types of the brain [2]. However, until recently, we did not have the technology to sequence the human genome at a single-cell level. Research Objectives: a) To identify cell-type specific non-coding gene regulatory changes associated with depression in a sex-specific manner, b) Multi-omics integration of single-nucleus epigenomic and transcriptomic datasets [3] generated from the same individuals c) Identifying cis-regulatory elements (CREs) and genes linked to MDD genetic variants with cell-type specificity. Methodology: Using high-throughput snATAC-seq [4] (Single Nucleus Assay for Transposase- Accessible Chromatin using Next Generation sequencing), we sequenced accessible regions of the genome in more than 200 thousand nuclei from the dorsolateral prefrontal cortex (dlPFC) of 44 individuals who during an episode of depression had died by suicide, and age- and sex-matched 44 healthy individuals who died by natural causes. Results: We found a majority of CREs with differential accessibility in MDD cases in microglial (58%) and deep-layer excitatory neuronal (21%) subtypes across both sexes. Early-response transcription factors (TFs), such as FOSL2 and JUN, showed increased binding in excitatory neurons, while canonical microglial transcription factors, such as SP1, showed a decreased binding in MDD cases compared to controls. We found that GWAS and snRNA-seq-identified MDD-associated genes significantly overlapped with the gene targets of our top TFs, suggesting that we identified key TFs regulating significant gene expression changes in MDD. Moreover, we found that excitatory neuronal cell-proportions were increased while astrocytic proportions were decreased in MDD cases across both snATAC-seq and snRNA-seq studies. Furthermore, we analyzed the moderation effect of sex on MDD pathology and found oligodendrocytic lineage cells showing a majority of sex-disease interactions. Interestingly, distinct TFs were found to be regulating these sex-specific changes in MDD. Moreover, using linkage disequilibrium score regression (LDSC), we found that MDD genetic variants were significantly enriched in the differential CREs of our top hits, mainly the deep-layer excitatory neurons and oligodendrocytes, but not in microglia. This suggested that CREs associated with genetic variants were limited to specific cell-types while microglia associated gene-expression changes mainly occurred through epigenetic mechanisms. Future Directions: Using a machine learning model, we will identify TF motifs disrupted by MDD genetic variants and then identify genes and biological pathways impacted by potentially causal genetic variants.

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IDENTIFYING EPIGENETIC VULNERABILITIES OF CHEMORESISTANT TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, characterized by early age of onset and poor prognosis. As there are few targeted therapies effective against TNBC, treatment typically defaults to chemotherapy. However, although tumours initially respond to chemotherapy, there is a high rate of recurrence due to chemotherapy resistance, leading to poor overall survival. This highlights a need for new therapeutic strategies to overcome drug resistance. Previous work in our lab has identified chromatin variants induced by metabolic reprogramming in paclitaxel-resistant TNBC cells. The resistance-associated chromatin variants exposed an epigenetic vulnerability to pharmacological inhibition of EZH2. It is currently unknown whether similar epigenetic vulnerabilities are present in TNBC harbouring resistance to different chemotherapeutic agents. To address this, we hypothesized that testing a broad selection of chemical probes targeting various chromatin modifiers would reveal epigenetic vulnerabilities across chemotherapy-resistant TNBC.
In our present work, we have established additional MDA-MB-436 cell lines that are resistant to two other chemotherapeutic agents, gemcitabine and cisplatin. We characterized growth, cross-resistance to other chemotherapy drugs, and response across a library of epigenetic drugs from the Structural Genomics Consortium (Toronto, ON). From the drug screen, we identified several candidate epigenetic probes that remain effective against chemoresistant TNBC which will be further investigated. In parallel, we will use chromatin accessibility to identify resistance-associated chromatin variants. Overall, our goal is to understand the epigenetic changes contributing to drug resistance and to identify novel therapeutic avenues.

USING MPRA TO UNDERSTAND THE FUNCTIONAL CORES OF TRANSPOSABLE ELEMENT ENHANCER ACTIVITY AND EVOLUTION IN PRIMATES

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Transposable elements (TEs) are known to be bound by transcription factors (TFs), to function as enhancers and to have been co-opted in multiple transcriptional programs during evolution. However, the evolution of TE enhancers and associated TF complexes remained largely elusive. Massively parallel reporter assay (MPRA) is a method that enables to simultaneously measure the regulatory activities of thousands of regulatory elements. In this study, using the MPRA approach along with a wealth of epigenetic data, we studied the functional cores of TE enhancers and evolution of their regulatory activity. We first identified MER11/34 and MER52 families with decreased and increased enhancer activities (ATAC-seq and H3K27ac), respectively, during the differentiation of human embryonic stem cells (ESCs) to neuronal progenitor cells (NPCs). After we determined the consensus functional regions of each candidate family, we retrieved target sequences among MER11/34/52 elements in human, chimpanzee and macaque genomes separately, and then submitted for the MPRA analysis. Through the phylogenetic analysis, we uncovered distinct sequence features underlying divergent regulatory activities within each family. Notably, we also identified functional regions and binding motifs that are strongly associated with the activities at single-nucleotide resolution. We found that SOXs and TFAP2s are the main factors correlated with the regulatory activity of MER11, however adjacent nucleotides may further impact the activity and lead to divergent patterns of active (e.g., H3K27ac and H3K4me1/2/3) and repressive epigenetic marks (e.g., H3K9me3) among individual elements. Lastly, we revealed the loss of regulatory activities in these LTR families during evolution. Our findings demonstrate the application of MPRA in studying TE enhancers at single-nucleotide resolution and elucidate the potential molecular mechanism of how TE regulatory activity evolved in primates.

EPIGENOMIC LANDSCAPE OF TRANSPOSABLE ELEMENT-DRIVEN LONG NON-CODING RNAS IN HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) has dismal clinical outcome but an incompletely understood epigenetic landscape. Transposable elements (TEs) have contributed to the regulatory activities of a diverse class of long non-coding RNAs (lncRNAs) in the genome. Recent studies have illuminated a link between transcriptional dysregulation of TEs and HCC development, but the functional capacities of TE-driven lncRNAs remain underexplored. Here, we interrogated the epigenomic and transcriptomic profile of TE-driven lncRNAs in HCC. We identified the cryptic activation of a HERVE-int-driven lncRNA (*lnc-HERVE-int*) that is silenced across normal tissues. Elevated *lnc-HERVE-int* expression was associated with more aggressive tumors, poorer patient survival and characteristic molecular subclasses of HCC, suggesting clinical relevance of *lnc-HERVE-int* in hepatocarcinogenesis. Notably, DNA hypomethylation at the HERVE-int promoter was correlated with but not sufficient for transcriptional induction of *lnc-HERVE-int*. This observation highlights additional mechanisms in suppressing HERVE-int activities. Together, our work provides an initial survey of the transcriptional activities of TE-driven lncRNAs in HCC and points to *lnc-HERVE-int* as a potential biomarker for the disease.

POLYCOMB REPRESSIVE COMPLEX 2 IS ESSENTIAL IN HUMAN TROPHOBLAST STEM CELLS

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The Polycomb Repressive Complex 2 (PRC2) is a key silencing complex that deposits the heterochromatic histone modification on Histone 3 Lysine 27 trimethylation (H3K27me3) and is commonly found at the promoters of repressed genes. To distinguish the functions of PRC2 in trophoblast and epiblast lineage, we mapped H3K27me3 in human trophoblast stem cells (hTSCs) and human embryonic stem cells (hESCs). We found that in hTSCs, this mark is found far higher global abundance and is spread throughout the genome instead of being localized at promoters as in hESCs. Additionally, we found that this repressive mark spans large domains of intermediate levels of DNA methylation called partially methylated domains (PMDs). These PMDs are a distinctive feature of the placental methylome and common in cancer cells, but less pronounced in somatic tissues and absent in hESCs. To determine the role of PRC2 in gene regulation in trophoblast, EED, a core component of PRC2 was knocked out in hTSCs using CRISPR-Cas9, generating H3K27me3 deficient cells which rapidly died. We performed RNA-seq to determine which genes and transposons were upregulated in these EED knockouts and to distinguish PRC2 targets in trophoblast and epiblast. To corroborate these results hTSCs were treated with EED226, a chemical inhibitor of EED. Slower cell growth was observed, and RT-qPCR confirmed the upregulation of PRC2 target genes. These results indicate the importance of PRC2 and H3K27me3 in gene regulation and trophoblast lineage.

OXYGEN SENSITIVE TRANSCRIPTION FACTOR GCM1 IS A MASTER REGULATOR OF DIFFERENTIATION IN TROPHOBLAST STEM CELLS

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The placenta develops alongside the embryo and nurtures fetal development to term. During the first stages of embryonic development, due to low blood circulation, the blood and ambient oxygen supply is normally very low (~1-2% O2) and gradually increases upon placental invasion. While a hypoxic environment is associated with stem cell self-renewal and proliferation,

persistent hypoxia may leave severe effects on differentiating cells that could be the underlying cause of placental disorders. In the placenta, human trophoblast stem cells (TSC) thrives in low oxygen, whereas differentiation of the progenitor cells, syncytiotrophoblast (STB) and extravillous trophoblast (EVT), is critically affected by hypoxic conditions. Our RNA-seq on 3 hTSC cell lines cultured at 2%-5%-20% O2 revealed oxygen sensitive transcription factors that are essential in hTSC differentiation. One of them is GCM1, which has very diminished levels at 2% O2. GCM1 (human Glial Cell Missing-1) is a transcription factor often associated with syncytialization of STBs and regulation of trophoblast cell invasion of EVT. Knock-out of GCM1 in TSC caused impaired EVT and STB formation and function, reduced expression of genes that respond to differentiation, and maintenance of self-renewal genes. Chromatin immunoprecipitation of GCM1 showed enrichment of GCM1 specific binding near key transcription factors upregulated upon differentiation, validating its function. Thus, hypoxia, via the downregulation regulation of GCM1, functions to maintains stem cell homeostasis and suppress EVT and STB terminal differentiation.

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 genomic 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 genomic 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 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.

NOVEL PHARMACOLOGICAL SENSITIVITY WITH 3D SPHEROIDS OF TRIPLE NEGATIF BREAST CANCER

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Breast cancer causes 25% of new cancer cases in Canadian women. Triple negative breast cancer (TNBC) is the most aggressive subtype with a high rate of metastatic relapse, and represents 15% of all breast cancer. TNBC is characterized by the lack of estrogen and progesterone receptors and do not exhibit HER2 amplification, which causes resistance to hormonal therapy or HER2 inhibitors. Even though conventional intensive treatments are used, patients still exhibit poor prognosis. Whereas new promising molecules were described, clinical trials often demonstrate lack of efficacy in patients, highlighting the limit of preclinical models.
Here, we propose to develop new TNBC 3D models to mimic patient's epigenome and transcriptome, which will be used for high-throughput drug screenings.
We established TNBC spheroid models from MDA-MB-231 and HCC-1806 cell lines. We demonstrated that these spheroids undergo a maturation process during long-term 3D culture, which peaked after 24 days. Interestingly, MYC oncogene and the histone methyltransferase EZH2 are overexpressed in 3D spheroids as shown in patients but lacking in monoculture cells, suggesting that our 3D spheroids may better recapitulate TNBC epigenome in situ.
By screening anticancer and epigenetic compound libraries, we highlighted that spheroids are significantly more resistant than their 2D counterparts. Additionally, we identified two drugs; a DNA methylation inhibitor and a PIM kinase inhibitor, which demonstrated significant pharmacological activities on our TNBC spheroids.
Overall, the development of new TNBC spheroids constitutes an innovative approach to study tumor biology and discover new specific drugs for this deadly form of breast cancer.

THE HISTONE H3.1 VARIANT REGULATES TONSOKU-MEDIATED DNA REPAIR DURING REPLICATION

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The tail of replication-dependent histone H3.1 varies from that of replication-independent H3.3 at the amino acid located at position 31 in plants and animals, but no function has been assigned to this residue to demonstrate a unique and conserved role for H3.1 during replication. We found that TONSOKU (TSK/TONSL), which rescues broken replication forks, specifically interacts with H3.1 via recognition of alanine 31 by its tetratricopeptide repeat domain. Our results indicate that genomic instability in the absence of ATXR5/ATXR6-catalyzed histone H3 lysine 27 monomethylation in plants depends on H3.1, TSK, and DNA polymerase theta (Pol q). This work reveals an H3.1-specific function during replication and a common strategy used in multicellular eukaryotes for regulating post-replicative chromatin maturation and TSK, which relies on histone monomethyltransferases and reading of the H3.1 variant.

DNA METHYLATION DYNAMIC IN MALE RAT GERM CELLS DURING GAMETOGENESIS

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In mammals, zygote formation and germline establishment are the two main developmental windows where the resetting of DNA methylation (DNAme), named epigenetic reprogramming, occurs. In the germline, there is near complete erasure of DNAme, followed by a sex-specific *de novo* DNAme. This establishes germline-specific gene expression profiles but also, in the longer term, a key component of the gametes epigenome that will guide, in part, embryo development. Male germline DNAme can be under the influence of environmental toxicants, leading to abnormal sperm epigenome and transgenerational epigenetic inheritance, but the initial molecular mechanisms linking exposure to an abnormal germline epigenome are still unknown. This is because we lack the comprehensive understanding of the establishment DNAme during gametogenesis, especially in rats, the preferred animal model in toxicology.
Here, we aimed to provide a developmental map of DNAme in the rat male germline throughout gametogenesis using the Agilent rat Methyl-Seq kit, targeting promoters, CpG islands, island shores, and GC-rich regions. To that end, germ cells were purified by fluorescent-activated cell sorting, using transgenic rats expressing GFP exclusively in germ cells, combined with DNA staining to gate for ploidy and nuclear compaction during spermiogenesis. We obtained 9 populations of developing germ cells (n=4 / stage): 1- proliferative gonocytes from gestational day 16 (GD16); 2: gonocytes entering the quiescent phase at GD18; 3: quiescent gonocytes at GD20; 4: spermatogonia at postnatal day 5 (PND5); 5: spermatids (stages 1-8); 6: spermatids 9-12; 7: spermatids 13-14; 8; spermatids 15-17; 9: epididymal sperm.
Our data showed that global DNAme level is minimal at GD18. The genomic regions being demethylated last (GD16 to GD18) were mostly intergenic yet, coding regions were enriched in genes involved in cell motility. DNAme is gradually restored until PND5 following three different patterns corresponding to distinct gene ontology enrichment. Interesting the regions being first remethylated and fast (GD18-GD20) were highly enriched in introns. Global DNAme patterns were almost similar between PND5 spermatogonia and spermatozoa, yet we identified many DMRs suggesting that spermiogenesis is a time for DNAme dynamic although with lower amplitude compared to perinatal stages. The highest number of DMRs was found when comparing R9-12 to R13-14 spermatids, revealing clusters of genomic regions, mostly intergenic, being hypermethylated at R9-12 and others, mostly introns, being hypomethylated at R13-14.
We have established an integrated map of the rat male germline methylome during gametogenesis, which will ultimately help identify epigenetic signatures of exposure to chemicals and test their specific sensitivity at critical stages of maturation.

OLIG2 MEDIATES A RARE TARGETABLE STEM CELL FATE TRANSITION IN SONIC HEDGEHOG MEDULLOBLASTOMA

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Functional cellular heterogeneity in tumours underlies their inability to be completely eliminated by therapy, causing relapse[1–4]. Previously, we demonstrated that the sonic hedgehog (SHH) subgroup of the childhood brain malignancy medulloblastoma (MB) has its growth rooted in a dysregulated developmental hierarchy, the apex of which is defined by characteristically quiescent *Sox2*-expressing stem-like cells[5]. By analysis of gene expression and chromatin accessibility patterns in specific cell states and functional MB compartments, we identified the basic helix-loop-helix transcription factor *Olig2* as governing the transition from the quiescent to the activated SOX2+ stem cell state, driving the generation of downstream neoplastic progenitors and differentiated progeny. Remarkably, by targeting this rare OLIG2-driven proliferative program genetically or with a brain penetrant small molecule inhibitor, stem cell emergence from quiescence and subsequent progeny formation is blocked. Further, early tumour formation as well as tumour regrowth from persister cells after therapy in vivo are also dramatically attenuated. We emphasise the critical importance of defining cancer lineage hierarchies and the mechanisms of cell state transitions to identify new cancer treatment approaches. We also demonstrate that targeting transition from quiescence to proliferation at the level of the tumorigenic cell could be a pivotal complementary MB treatment strategy.

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FUNCTIONAL MUTATIONS IN DNA METHYLTRANSFERASE 3A (DNMT3A) LEAD TO ALTERED GENE EXPRESSION IN INDUCED-PLURIPOTENT STEM CELLS THAT IS INTENSIFIED DURING NEURAL SPECIFICATION.

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DNMT3A activity is required during development for establishing DNA methylation, a stable epigenetic mark crucial for the regulation of gene expression. During brain development, DNMT3A is strongly expressed to drive and regulate proliferation and differentiation of neuronal populations. Rare heterozygous mutations in functional DNMT3A domains cause an overgrowth intellectual disability syndrome called Tatton-Brown-Rahman Syndrome. Currently, we do not know how functional mutations in human *DNMT3A* can impair neurodevelopment. We postulate that pathogenic heterozygous *DNMT3A* mutations will lead to DNA methylation programming defects that will alter gene expression profiles during neurogenesis.
To investigate such events, we derived 2 different induced-pluripotent stem cell lines from patients carrying a single mutation in the functional methyltransferase domain of DNMT3A. We differentiated these induced pluripotent stem cells into neural progenitors and performed RNA sequencing and bioinformatics analyses to identify gene expression perturbations highly enriched during neurogenesis and neurodevelopment processes. My current results show that transcriptional errors are triggered during the reprogramming process into induced pluripotent stem cells, without affecting associated pluripotency characteristics. I also show that during the initial steps of neural lineage specification, the impact of *DNMT3A* mutations on gene expression is exacerbated.
I observe that expression alterations in mutated neural progenitor cells are associated with genes that are key for brain development and cell fate commitment for instance and related to characteristic phenotypes observed in Tatton-Brown-Rahman Syndrome. My data suggests that *DNMT3A* mutations lead to dysregulation of neuronal subtype cell fate during the neurogenesis process.

EVALUATING ENHANCER SEQUENCE RULES IN MOUSE EMBRYONIC STEM CELLS USING PREDICTIVE MODELS OF GENE EXPRESSION

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Enhancers are a class of cis-regulatory elements that are essential for organism development, capable of driving gene expression in a cell-type specific manner. Despite this important role, the rules that govern enhancer function have eluded researchers. A variety of theories have been proposed to explain the relationship between sequence and function, generally focusing on the presence of transcription factor binding sites (TFBS) and their arrangement within the enhancer sequence. These theories disagree on the extent to which this grammar influences enhancer function. In recent years, deep learning has been applied to train neural networks to predict gene expression and various functional genomics assays from DNA sequence. Are these neural networks learning the enhancer code? If so, can we design experiments to derive this code from them? To investigate these questions, we have trained a neural network to predict enhancer activity from DNA sequence in mouse embryonic stem cells (mESC). We find that, while the neural network attributes importance to many well-known pluripotent transcription factor motifs, such as those for Oct4, Sox2, and Klf4, it also identifies numerous other motifs that contribute to enhancer activity. This breaks from a widespread belief where a select few master regulators are sufficient to explain enhancer activity and is consistent with our recent observations regarding the importance of TFBS diversity. Additionally, the neural network identifies experimentally validated binding sites for Sp1, Esrrb and Sox3 in the SOX2 SRR111 enhancer, demonstrating that the neural network is

learning genuine enhancer features. Finally, we explore whether synthetic sequences can be designed to extract the features and rules that are being learned by the neural network. Initial results demonstrate that sequences with heterotypic motifs create strong enhancers with low variance in activity, whereas sequences with homotypic motifs typically create weak enhancers with high variance in activity. Deciphering the sequence rules neural network models use to predict enhancer function in mESC will provide us with a greater understanding of how organisms utilize gene regulation to control early development.

O-GLCNACYLATION, AN EMERGENT EPIGENETIC MODIFICATION INVOLVED IN SEPSIS

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Background: The O-GlcNAcylation (O-GlcNAc) is an emerging post-translational modification which occurs on serine and threonine residues. This protein modification is notably involved in cell survival and stress response but also at every step in the cascade of effectors implicated in both transcription and epigenetic regulation. We previously demonstrated that stimulated O-GlcNAc levels is beneficial in adult rats in response to infection (ie. sepsis). Considering that O-GlcNAc levels are physiologically higher in the young, we hypothesize that the O-GlcNAc stimulation may have a different effect in young rats in sepsis. **Aim:** Evaluate if O-GlcNAc stimulation is beneficial to sepsis response in young rats and identify the associated mechanisms. **Methods:** Endotoxemic shock was induced in 28 days old rats by lipopolysaccharides injection (E. Coli O111:B4, 20mg.kg^{-1} - LPS) and compared to control rats (NaCl 0.9% - CTRL). One hour after lipopolysaccharides injection, rats were randomly assigned to no therapy (LPS), fluidotherapy (NaCl 0.9%, 10mg.kg^{-1} - LPS+R) supplemented or not with NButGT (10 mg.kg^{-1} - NButGT) to increase O-GlcNAc levels. 2 hours later, physiological functions and plasmatic markers were measured and used in adapted Pediatric RISk of Mortality score (PRISM score). The impact of treatment on survival was evaluated (n=64). Cardiac O-GlcNAcylated proteins were mapped by untargeted mass spectrometry and genes transcription evaluated via 3' SRP analysis. **Results:** LPS induced a shock (mean arterial pressure (MAP): CTRL: 67.2±1.9; LPS: 50.7±2.1; mmHg; $p<0.05$), altered biological parameters (lactates: CTRL: 3.92 ± 0.26; LPS: 6.42 ± 0.45; mmol.l^{-1}; cardiac troponin T: CTRL: 19.7 ± 4.0; LPS: 45.4 ± 11.4; ng.l^{-1}; $p<0.05$) and PRISM score ($p<0.05$). LPS+R had no beneficial effect while NButGT improves MAP (NButGT: 72.2±4.0 mmHg; $p<0.05$), PRISM score ($p<0.05$) and the median survival (NButGT: 36.0; LPS+R: 13.65; hours; $p<0.001$) compared to LPS+R treatment. A total of 33 proteins are differentially O-GlcNAcylated in all groups. Among them, 60% are involved in metabolism and metabolic pathways. Mass spectrometry identifies the ATP-citrate lyase as the only protein less *O*-GlcNAcylated in the NButGT group; interestingly its phosphorylation in position 447 and 451 are unchanged, suggesting that O-GlcNAcylation does not impact these phosphorylation sites. LPS injection promotes cytokines pathway. Yet, the mRNA expression was not impacted 2 hours after treatment. **Conclusion:** Acute O-GlcNAc stimulation improves outcome in young septic rat. Stimulate the O-GlcNAcylation increased cardiac O-GlcNAcylated proteins notably involved in metabolic pathways. Interestingly, the transcription is not impacted by the O-GlcNAc stimulation. The impact of *O*-GlcNAcylation on the ATP-citrate lyase is an interesting avenue. Evaluate the modulation of the O-GlcNAc mark on different transcriptional and epigenetic regulators could be also a potential perspective to unravel its role in the sepsis.

RESEARCHER PERSPECTIVES ON ETHICS CONSIDERATIONS IN EPIGENETICS: AN INTERNATIONAL SURVEY*

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Over the past decade, bioethicists, legal scholars and social scientists have started to investigate the potential implications of epigenetic research and technologies on medicine and society. There is growing literature discussing the most promising opportunities, as well as arising ethical, legal and social issues (ELSI). This paper explores the views of epigenetic researchers about some of these discussions. From January to March 2020, we conducted an online survey of 189 epigenetic researchers working in 31 countries. We questioned them about the scope of their field, opportunities in different areas of specialization, and ELSI in the conduct of research and knowledge translation. We also assessed their level of concern regarding four emerging non-medical applications of epigenetic testing—i.e., in life insurance, forensics, immigration and direct-to-consumer testing. Although there was strong agreement on DNA methylation, histone modifications, 3D structure of chromatin and nucleosomes being integral elements of the field, there was considerable disagreement on transcription factors, RNA interference, RNA splicing and prions. The most prevalent ELSI experienced or witnessed by respondents were in obtaining timely access to epigenetic data in existing databases, and in the communication of epigenetic findings by the media. They expressed high levels of concern regarding non-medical applications of epigenetics, echoing cautionary appraisals in the social sciences and humanities literature.

ALLELE-SPECIFIC METHYLATION EDITING OF IMPRINTING GENES

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Following fertilization, a major reprogramming wave removes most DNA methylation signatures across the genome to instigate the embryonic developmental program. Through mostly unknown mechanisms, parent-of-origin-specific methylation marks on one allele of imprinted genes (e.g., *Igf2*: Insulin-Like Growth Factor 2/H19 locus) are able to escape the embryonic reprogramming wave and retain their profiles via maintenance by the DNA methyltransferase 1 (DNMT1) family proteins. Failing to maintain proper genomic imprinting patterns during embryonic reprogramming leads to neurodevelopmental disorders characterized by developmental delay. It is still unclear how embryonic cells are able to re-establish DNA methylation profiles across most of the genome following a temporary lack of DNA methylation maintenance, while other regions such as imprinted loci become permanently dysregulated.
Published and current data from the McGraw lab show that, for *Igf2*/*H19* imprinting loci, a transient loss of DNA methylation maintenance in mouse embryonic stem cells (mESCs) leads to permanent loss of DNA methylation, dysregulation of histone marks, and an increase in gene expression. My current results show that when I target such imprinted region in the same mESC using non-allele-specific epigenome editing system I was able to remethylate the regulatory region of H19 that had DNA methylation loss and significantly lower the expression more than half-way back towards normal levels, confirming that the epigenome editing system we are working with works correctly.
What remains to be elucidated is whether we can permanently re-establish normal profiles of DNA methylation and subsequent histone modifications on imprinted (allele-specific) sequences following the loss of their DNA methylation imprinting status. To accomplish this, we are using our epigenome editing systems in combination with mESCs derived from the backcross of two different strains (B6xCAST7) to achieve allele-specificity. To induce the loss and re-establishment of DNA methylation on *Igf2*/*H19* imprinting loci we are performing lentiviral transduction in mESCs to express the protein complex with the allele-specific gRNAs. Finally, to delineate the adaptation in histone modifications and gene expression, we will define if similar perturbations in histone modifications and gene expression (similar to our preliminary results) are observed following the targeted allele-specific loss of the imprinting status at the *Igf2*/*H19* locus.
In conclusion, this project will further the understanding of the fundamental complex biochemical mechanisms of epigenetic regulation that drive allele-specific gene expression in embryonic cells. It will reveal how such mechanisms are involved in imprinting maintenance during early embryogenesis and will elucidate possible causes leading to imprinting developmental disorders.

EPIGENETIC PLASTICITY IN DEVELOPMENT AND CANCER

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Epigenetics helps define current cell states, yet also shapes how cells respond to external cues such as differentiation or stress. The epigenetic plasticity of a cell describes how flexible this regulation is. Early embryonic cells are highly plastic in that they can generate all adult cell types. As development progresses, this plasticity is lost as normal healthy adult cells are locked in their identity. Crucially, aberrant reactivation may contribute to pathologies such as cancer.
The epigenetic plasticity of cells is tightly controlled and regulated in part by epigenetic priming factors. These establish a permissive epigenetic landscape to enable future transcriptional changes. However, we know little of the identity of epigenetic priming factors and their mode of action. I will present our work uncovering new molecular signatures of epigenetic plasticity. Using these signatures, we have performed screens to identify epigenetic priming factors. This has identified the small heterodimerising nuclear proteins *Dppa2* and *Dppa4* as the first epigenetic priming factors. Dppa2/4 are required to maintain both H3K4me3 and H3K27me3 at a set of developmentally important bivalent promoters. As a consequence of losing bivalency, these promoters gain DNA methylation and can no longer be effectively activated during differentiation. These epigenetic changes are reversible on reintroduction of Dppa2/4 suggesting that Dppa2/4 are required to actively target and maintain the epigenetic landscape at these developmental genes in pluripotent cells.
We are now exploring how these important gatekeepers of early embryonic cell fate transitions may be hijacked in cancers to promote cell plasticity and facilitate acquisition of new identities and functions. By discovering the principles driving epigenetic plasticity in development, we can further our understanding of how this goes awry in cancer, leading to new areas for therapeutic intervention.

STUDYING RNA-MEDIATED TRANSCRIPTIONAL REGULATION BY THE FORMATION OF RNA:DNA TRIPLEXES

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The formation and spreading of breast cancer metastases, where cells transition from an epithelial “static” state to a mesenchymal “mobile” state, is a dynamic process characterized by changes in gene expression driven by key transcription factors and chromatin remodeling complexes. It is now well established that RNA is a critical component of chromatin and plays a role in regulating transcriptional outputs. Distinct RNA transcripts can be targeted to specific loci either through protein interactions as part of ribonucleoprotein complexes or by binding directly in a sequence specific manner to DNA, either by forming standard Watson-Crick base pairs with one of the DNA strands (R-loops) or by forming non-canonical Hoogsteen base pairs with the DNA double helix (RNA:DNA triplexes). These interactions allow the targeting of RNAs to genomic loci, while other parts of the RNA can help recruit protein partners to organize a local regulation of transcription. Since only a handful of RNAs have been experimentally shown to form functional RNA:DNA triplexes, we

still lack a good understanding of the amount of RNA-DNA triplexes formed in cells, their roles, and their dynamics in processes such as the epithelial-to-mesenchyme transition (EMT).
To identify triplex-forming noncoding RNAs (TF-ncRNAs) involved in EMT, we use the MCF10A breast epithelial cells treated with TGFb-1 to simulate mesenchymal transition. We performed time-course RNAseq and ATACseq to identify noncoding RNAs involved in early and late stages of EMT, as well as differentially opened, putative triplex-forming genomic regions. We identified both known and novel unannotated transcripts which are dynamically regulated during EMT and, in parallel, have biochemically purified triplex-forming RNA transcripts. These are being targeted for CRISPRa/i loss- and gain-of-function assays to characterize their function in the EMT process. Identification of TF-ncRNAs will shed new light on the role of RNA in chromatin regulation and will uncover a novel layer of genome and transcriptional regulation during metastasis formation.

TEMPORARY LOSS OF DNA METHYLTRANSFERASE 1 (DNMT1) CAUSES MULTILAYERED EPIGENETIC INSTABILITY IN EMBRYONIC STEM CELLS

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Early embryonic development represents a particularly vulnerable window as the epigenome undergoes extensive reprogramming to initiate the developmental program. The maintenance of DNA methylation by DNA methyltransferase 1 (DNMT1) is of utmost importance during this stage and for regulating cell fate transitions in later development. More recently, DNMT1 has also been shown to have a non-canonical role in modulating histone modifications. Complete absence of DNMT1 is embryonic lethal in mice, while insufficient activity results in disorganized cell differentiation and pronounced cell death of specialized cells in both mice and humans, as well as implantation failure of the mouse embryo. DNMT1 activity can be directly altered by genetic mutations and environmental factors, such as alcohol or neurotoxins, or indirectly altered via dysregulation of its many mechanistic partners and lead to heritable epigenetic errors. Although DNMT1 plays a crucial role in embryonic development, we remain remarkably unaware of its precise mechanisms and how it can be involved in the inherited dysregulation of epigenetic marks. Here, using a transgenic mouse embryonic stem cell model (mESC) in which we can robustly control *Dnmt1* expression, we sought to decipher the interplay between the canonical and non-canonical roles of *Dnmt1* and how dysregulation of these mechanisms may hinder differentiation of early embryonic cells. We hypothesized that temporary inactivation of *Dnmt1* in mESCs would lead to heritable instability on multiple epigenetic layers (DNA methylation, histone modifications) which would disrupt gene expression patterns and hinder differentiation potential. We show that after the inactivation and rescue of *Dnmt1*, millions of regions across the genome can recover their DNA methylation profiles; however, various regions show heritable DNA methylation dysregulation. The temporary loss of *Dnmt1* also prompts permanent and substantial genome-wide remodeling of histone modifications (H3K4me3, H3K27ac, H3K27me3, H3K9me3, H3K4me1) as well as gene expression dysregulation of many pioneer factors (e.g., *Trp53* and *Sox*, *Hox*, *Fox*, *Gata* gene families), cellular differentiation pathways (e.g., BMP, WNT) and genes involved in epigenetic mechanisms (e.g., *Kmt2e*, *Kdm5d*/*4b*/*7a*, *Dnmt3b*). Upon differentiation of mESCs towards ectodermal, mesodermal, and endodermal germ layer lineages, these embedded inherited epigenetic errors induce lineage-specific and pan-lineage gene expression errors. The mesoderm appears to be the most specifically affected, having nearly 4000 dysregulated genes; we see downregulation of mitotic cell cycle and chromatin organization pathways and upregulation of genes involved in cell death. Overall, this project will elucidate the fundamental mechanisms of DNMT1 in early development and contribute to establishing a molecular basis of cell-to-cell transmission of epigenetic errors; improving our mechanistic understanding of how initial perturbations in the embryonic epigenetic program emerge and persist at specific loci following an insult, and how they affect cell fate transitions and specifications.

DEFINING ENDOTHELIAL REGULATORY NETWORKS IN A MOUSE MODEL OF ATHEROSCLEROSIS.

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder worldwide, and is characterized by the accumulation of neutral lipids, resulting in cellular damage and an injury response that leads to fibrosis. NAFLD has also been demonstrated to be an independent risk factor of atherosclortic cardiovascular disease (CVD); with CVD being the primary cause of death for NAFLD patients. Endothelial cells comprise the dominant cell type of vascular walls and are known to be key players in CVD. The population of hepatic endothelial cells consists predominantly of liver sinusoidal endothelial cells (LSECs), which maintain the homeostasis of the sinusoidal niche through endocytosis and the production of angiocrine factors. In the context of cellular damage LSECs are responsible for promoting angiogenesis, releasing pro-inflammatory cytokines, and recruiting inflammatory monocytes to the liver via cell surface proteins. In this study we set out to identify how LSEC gene regulatory elements are modulated during atherosclerosis. Identifying the genes and the cis regulatory elements (CREs) that control the endothelial response to hepatic fatty buildup will allow us to discover genetic variants that contribute to chronic liver disease and vascular disease. To this end, we characterized the liver phenotype in an atherosclerosis mouse model (*Ldlr*$^{-/-}$ mice). Bulk liver RNA-seq and qPCR revealed striking upregulation of inflammatory pathways and pro-inflammatory cytokines following 4 weeks of high cholesterol diet (HCD), accompanied by an influx of recruited monocytes and depletion of resident macrophages (Kupffer cells). Intriguingly, this inflammatory phenotype was fully reversible when mice were returned to a control diet. Transcription factor (TF) footprinting results from ATAC-seq performed on isolated LSECs from mice fed HCD or control diet

revealed an enrichment in binding of the NF-κB, KLF and AP-1 families in HCD mice with a corresponding loss in binding of the ETS family of TFs, which are key for maintaining endothelial identity. This change in binding dynamics is similar to our prior *in vitro* work examining changes in endothelial regulatory pathways following Ets-related gene (ERG) knockout. We hypothesize that TNF-alpha induced activation of NF-κB subunits result in the dysregulation of ERG and is a defining event in the LSEC inflammatory phenotype. Moving forward we will test this hypothesis through the use of an endothelial specific inducible RELA knockout mouse. These results highlight significant and rapid alterations in the composition of the immune cell population of the liver and the chromatin environment of endothelial cells following hepatic fatty buildup, and hint at a central role of the NF-κB pathway in driving the endothelial response to fatty liver.

ELUCIDATING THE IMPACT OF HISTONE MUTATIONS IN CANCER

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Histone proteins are a key epigenetic platform for modulating the accessibility and compaction of the human genome, affecting the replication, repair, and expression of DNA. Recent studies have demonstrated that some histone missense mutations are capable of perturbing regular histone function and promoting the development of phenotypically distinguishable cancers. Nonetheless, most histone mutations observed in cancer remain poorly documented regarding their potential to promote oncogenesis. It is unknown as to which mutations may increase the risk of cancer development and what mechanisms they may use to meet this end. Moreover, the impacts that these mutations may have on patient prognosis and clinical outcomes remain elusive.
To characterize histone missense mutations in these regards, we have gathered whole-exome sequencing data for over 13,000 tumor samples and the accompanying clinical data for the patients who harbor said tumors. We have identified over 2,800 distinct histone missense mutations from five types of histones, across 2,029 patients. Using novel computational methods, we have predicted those mutations that can act as drivers of cancer development, within the context of the 65 cancer types in which they are observed. In total, 274 histone missense mutations have been identified as probable drivers in our surveyed cancer types. Through leveraging histone interaction networks, we have been able to identify specific interactions and binding interfaces which are impacted by histone mutations. We note that histone mutations are generally enriched at histone-histone and histone-DNA interfaces, whereas our predicted driver mutations are only found to be enriched at histone-histone interfaces. Through leveraging computational methods, we have also quantified the ability for individual histone missense mutations to disrupt the interfaces that they overlap. Extending beyond the biophysical perspective, we have found that some of our predicted driver mutations may be associated with lower overall survival in patients of specific cancer types.

CHROMATIN STATE ANNOTATION OF CIS-REGULATORY ELEMENTS IN HUNDREDS OF HUMAN CELL TYPES USING SEGWAY WITH APPLICATIONS TO DISEASE ASSOCIATION

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Segmentation and genome annotation (SAGA) methods such as Segway are widely used to gain an integrative understanding of genomic activity. These methods take as input several epigenomics tracks such as histone modifications and DNA accessibility for a cell type, and output high-resolution annotations that assign each genomic region to a category of activity. Segway performs this task in two steps. The first step is the unsupervised high-resolution partitioning of the genome into several chromatin states based on the input from the epigenomics tracks. In this step, each segment is annotated by a state label that corresponds to a type of genomic activity. In the second step, these labels are interpreted to genomic functional terms, such as "enhancer" and "promoter". We use an automated process for the interpretation step, where the functional terms for each label are identified based on a set of features from the previously annotated samples.
In this work, we produced a new set of Segway annotations for hundreds of human tissues and cell-types with improvements to the automated interpretation process. The new set of annotations allows us to investigate the role of disease-associated variants in the relevant cell-types or tissues and identify their effect on gene regulatory mechanisms.
The outcome of this project will be reference Segway annotations for all ENCODE cell types, where annotations assign each genomic locus to an activity type from the controlled vocabulary of chromatin states. These annotations identify all observed cis-regulatory elements in the genome along with their pattern of activity across cell types.

DNA METHYLATION SIGNATURES FOR PREDICTION OF EARLY-ONSET PREECLAMPSIA IN THE HUMAN PLACENTA

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Introduction: Preeclampsia (PE) is a pregnancy complication characterized by maternal hypertension that affects 2-8% of pregnancies worldwide and increases the risk of perinatal complications for both mother and fetus. Placental insufficiency is associated with PE, although the precise molecular mechanisms vary depending on the stage of pregnancy at which the disorder develops. PE that develops earlier in gestation is often more severe and more likely to co-occur with fetal growth restriction (FGR). Thus, PE is often clinically subdivided based on the gestational age (GA) at onset: early-onset (EOPE, <34 weeks) and late-onset (LOPE, >34 weeks). However, using a gestational

age cut-off to discriminate the severity of PE is limited by the heterogeneous nature of this disorder. For example, human placentae from pregnancies complicated by EOPE present widespread DNA methylation (DNAme) changes not seen in LOPE, that are partly replicated across studies. We hypothesise that placental DNAme signatures can be used to develop a probability score that considers PE in a spectrum of severity.
Methods: Illumina HM450K placental data from 4 public datasets (N=83; 42 EOPE, 41 nPTB) were used for model training, and two datasets (N=48, 38 EOPE, 10 nPTB) were reserved for validation. Samples in both training and testing cohorts were divided into two classes: 1) EOPE, defined as delivery < 34 weeks of GA, and 2) normotensive preterm (nPTB), defined as samples without PE and/or IUGR of less than 37 weeks of GA. Data for the training and testing cohorts was independently pre-processed, normalized, and batch-corrected for the dataset effect. In addition, a third cohort (N=243) of publicly available Illumina HM450 data was independently assembled, pre-processed, normalized, and reserved for additional validation of the model. Sparse partial least squares discriminant analysis (sPLS-DA) was selected as an effective 'omics machine learning method given its simultaneous dimension-reduction and feature selection capability. Six iterations of repeated 3-fold cross-validation were performed to find the optimal number of components and variables per component for the sPLS-DA model.
Results: Cross-validation yielded a one-component final model with 64 predictive CpGs. Testing on the validation cohort yielded an accuracy of 77% and a positive predictive value of 86%. Interestingly, 5 nPTB samples with co-occurring FGR were classified as EOPE. FGR and EOPE have been previously shown to have an overlapping DNAme signature. Further, the 6 EOPE samples that were misclassified as nPTB had significantly lower probabilities of belonging to the nPTB class (mean probability=0.54) than the 32 EOPE samples that were correctly classified as EOPE (mean probability=0.13).
Conclusion: A DNAme-based classification model may be able to detect samples that have a DNAme signature associated with PE that develops earlier in gestation and is linked with FGR. This model can also be used to assign a probability score on a continuum that may better reflect the underlying pathology.

dCypher®: A HIGH-THROUGHPUT METHOD FOR THE ASSESSMENT OF CHROMATIN INTERACTING PROTEINS

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The pattern of histone modifications is recognized though epigenetic regulators through evolutionary conserved binding domains (aka Readers), which are recruited to nuclear complexes at specific genomic loci. Isolated readers and histone peptides have been frequently used to define [Reader-histone PTM] specificity. However, this is a reductive approach and assumes that PTM readout is unaffected by any high order factors. To assess the limitations and drawbacks of a reductive approach, EpiCypher® has developed dCypher® a high throughput discovery platform for the rapid screening and detailed characterization of chromatin interactors including Readers, antibodies, and enzymes. This platform is based on a no-wash bead-based proximity assay (i.e. AlphaScreen®) and utilizes comprehensive libraries of single and combinatorial modified histone peptides (>287) and designer nucleosomes (>100) encompassing ~100 unique PTMs on the four core histones, as well as several histone and DNA methylation variants. dCypher was employed to investigate the histone peptide and nucleosome binding interactions of the tandem PHD-BD within BPTF, part of the Nucleosome Remodeling Factor complex. We show that BPTF histone PTM specificity is in fact dependent on nucleosome context. Further we demonstrate that the *in vitro* specificity of a tandem reader for nucleosomes is recapitulated in a cellular context using CUT&RUN. These findings demonstrate the critical importance of using nucleosome substrates to garner insights into *in vivo* binding mechanisms.

TOWARDS ROBUST ANNOTATION OF CHROMATIN STATES

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International collaborations such as ENCODE, CEEHRC, and IHEC have been collecting, organizing, and publishing numerous epigenomic datasets. Segmentation and genome annotation (SAGA) algorithms such as ChromHMM and Segway are widely used to annotate chromatin states using epigenomic datasets. SAGA algorithms rely on probabilistic graphical models and take as input a collection of genomics datasets, partition the genome, and assign a label to each segment such that positions with the same label have similar patterns in the input data. They output an annotation that assigns to each genomic position its annotated activity, such as Enhancer, Promoter, etc. SAGA annotations enable researchers to easily and intuitively understand the activity of any genomic position in any assayed cell type and thus are a key output of large-sale epigenome mapping projects.
Despite the widespread use of SAGA methods, there is currently no principled way to evaluate the statistical significance of their label assignments. In this study, we aim to apply principles of reproducibility analysis to assess the statistical confidence that is to be attributed to SAGA annotations. This principled approach enables the evaluation of SAGA annotations independent of the choice of SAGA method, intended application, or input data.
In this research project, performing an array of investigations, we comprehensively evaluate several hypotheses:

1. Irreproducibility of SAGA annotations across replicated experiments stems from various experimental or technical sources such as data processing, model training, or even random initialization of models.
2. Reproducibility differs across functional elements in the genome; certain chromatin states, with a distinguishable pattern of histone modification marks, are more likely to be reproduced.
3. Reproducibility depends upon the number of annotated chromatin states, as a higher number of labels results in over-

splitting functional element categories and reduced overall reproducibility.
4. Posterior probabilities of SAGA label assignments can be insightful confidence estimates as they are correlated with reproducibility.
5. Through qualitative and quantitative reproducibility analysis, we can determine if certain SAGA annotations classes are reliably reproducible.

Our results indicate bounds on the robustness of current SAGA annotations. Thus, effectively using chromatin state annotations from existing SAGA Methods requires understanding the circumstances in which they lead to reproducible output. This research will lead to approaches that can yield robust, statistically significant SAGA annotations.

PREDICTION OF METADATA FROM EPIGENOMIC DATA ENABLED VALIDATING/CORRECTING/COMPLEMENTING IHEC EPIATLAS PROJECT AND OVER 20,000 CHIP-SEQ UNIFORMLY REPROCESSED PUBLIC DATASETS

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Over the last decade, major initiatives such as the International Human Epigenome Consortium (IHEC) that includes ENCODE, BLUEPRINT and CEEHRC have generated thousands of high-quality reference epigenomic datasets in a large variety of assays and cell types. In parallel, raw data is also accumulating in public databases (DB) such as GEO (Gene Expression Omnibus), containing ~35k human ChIP-Seq samples related to one of the six IHEC's core histone modifications (and their corresponding input samples). Since the metadata hosted in GEO has no imposed standardization, initiatives such as ChIP-Atlas, NGS-QC and Cistrome have tried to harmonize the GEO metadata for thousands of samples (~23k present >1 DB). Importantly, ~7.5% of the target (also called assay) metadata interpretation is different between these initiatives. Based on our previous works developing the epiGeEC (epigenomic Efficient Correlator) tool, we hypothesized that using epigenomic data to predict their metadata was feasible and desirable.

To test this hypothesis, we used the harmonized ChIP-seq EpiAtlas data generated by the IHEC Integrative Analysis working group to develop and train EpiLaP (Epigenomic Labeling Predictor), a neural network having accuracy and precision >98% to predict ChIP-Seq targets. EpiLaP detected with high confidence a few sample inversions and mislabels within the highly curated EpiAtlas metadata, supported by visual inspections through a genome browser and by other machine-learning models such as random forest, logistic regressions and regularized gradient boosting that we also trained. We then used EpiLaP on the 20,917 ChIP-Atlas uniformly reprocessed samples and were able to resolve with high confidence the interpretation disagreement for the vast majority. Even more interesting, ~9% of the ~13.5k samples with a metadata interpretation consensus among DBs and with a high confidence EpiLaP prediction were identified as mislabeled (confirmed by other models and visual inspections).

In addition to the validation/correction of the ChIP-Seq target metadata, considering that <0.01% of the GEO data contained metadata attributes such as sex and biomaterial type, we also trained EpiLaP and other models on the EpiAtlas harmonized metadata and data to complement with high confidence the original metadata information of >75% of the public samples.

INVESTIGATING THE EPIGENETIC REGULATION OF INTRA-TUMOUR METABOLIC HETEROGENEITY IN TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is a highly heterogeneous subtype of breast cancer. Along with high rates of chemoresistance, intra-tumour heterogeneity of TNBC makes therapeutic targeting challenging, contributing to the poor outcome of TNBC patients. Tumour progression is characterized by the reciprocal regulation of metabolic changes and epigenetic reprogramming that support tumour proliferation and adaptation to stress. We hypothesize that 1) the intra-tumour oxygen and nutrient fluctuations lead to dynamic and heterogeneous reprogramming of epigenetic landscapes within TNBC, and 2) this epigenetic reprogramming provides heterogeneous metabolic gene regulations that support the growth and adaptation of TNBC. Using multiplex immunofluorescence staining and spatial transcriptomics analysis of TNBC patient-derived xenografts (PDXs) and matched patient samples, we have identified two metabolically distinct cancer cell populations within TNBC that exhibit mutually exclusive spatial localization patterns and different distances to tumour vasculatures. We show that these two intra-tumour metabolic zones are characterized by the different levels of trimethylation of lysine 27 on histone 3 (H3K27me3), which regulates chromatin accessibility and gene expression. Accordingly, inhibition of Enhancer of Zeste Homologue 2, the methyltransferase responsible for H3K27me3, results in transcriptional signatures associated with amino acid metabolism, including nitrogen metabolism in TNBC models. This suggests a spatiotemporal H3K27me3-mediated regulation of nitrogen metabolism in TNBC. This project will decipher epigenetic reprogramming that modulates intra-tumour metabolic heterogeneity and metabolic flexibility of TNBC.

POLYCOMB REPRESSION IN SKELETAL MUSCLE STEM CELLS

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Skeletal muscle stem cells (commonly known as satellite cells) are essential adult stem cells for maintaining the integrity of muscle tissues over the course of an organism's life. When injuries to muscles occur, the satellite cells are activated converting into a new cell type known as myoblasts, capable of expanding their population to generate sufficient precursor myonuclei to regenerate the damaged muscle fibers. The activated myoblasts continue to expand their population, until molecular queues which direct the cells to self-renew back to the stem cell population, or to differentiate into myocytes and subsequently fuse into the damaged muscle fibers occur. To govern the changes to these states, precise epigenetic regulation is required to ensure that the changes between these cell states happen at the appropriate times, and to ensure that the correct number of cells self renew or differentiate. Many different epigenetic regulators are involved, some of which are the Polycomb complexes.

The Polycomb complexes are broken down into two major groups, being the Prc1 and Prc2 complexes both of which can be broken down into further subgroups which can have specific functions within cells. Two major roles of the Polycomb complexes are the deposition of the histone modifications H2AK119ub1 and H3K27me3 catalysed by the Prc1 and Prc2 complexes respectively. The Prc1 complex also has major roles in driving chromatin compaction through the polymerization of Prc1 complexes. This can create phase separation loci within the nucleus and the formation of Polycomb domains, driving strong gene repression over these loci.

In the context of skeletal muscle regeneration, the Polycomb proteins regulate aspects of the changes between these states. Roles of the Prc2 complex in preventing the expression of late myogenesis genes have been previously discovered, however insufficient studies have been performed to determine the involvement of the Prc1 complex in myogenesis. Our work seeks to resolve the question as to what the role of the Prc1 complex is during myogenesis. We analysed the role of the Prc1 complex in regulating the process of the myoblasts differentiating *in vitro*, using enzymatic inhibitors for the H2AK119ub1 activity of the Prc1 complex. This resulted in a defect in differentiation, with fewer myonuclei being incorporated into myofibers. These results suggest that there could be a defect in the fusion of the differentiating muscle precursors, although the precise mechanism has not yet been determined.

Apart from the enzymatic activity of the complex, we also wish to determine the role of the remainder of the complex. We are using as CRISPR/Cas9 strategy to derive controlled deletions of the complex to determine what stages of myogenesis are disrupted when the complex is disrupted. This will be done both *in vivo* and *in vitro* and will allow us to understand the full role of the Prc1 complex in myogenesis.

PERIPHERAL BLOOD DNA METHYLATION AND NEUROANATOMICAL RESPONSES TO HDACI TREATMENT THAT RESCUES NEUROLOGICAL DEFICITS IN A KABUKI SYNDROME MOUSE MODEL

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Background: Recent findings from mouse models of Mendelian neurodevelopmental disorders (NDDs) strongly support the potential use of postnatal therapies to improve neurocognitive deficits. As several of these therapies move into human clinical trials, the search for biomarkers of treatment efficacy has become a priority. Kabuki syndrome (KS) is an NDD caused by pathogenic variants in *KMT2D*, a histone-lysine methyltransferase. A unique DNA methylation (DNAm) signature is detectable in blood of KS patients, likely resulting from epigenetic crosstalk between DNAm and histone methylation directly affected by KMT2D. A potential postnatal treatment of KS has emerged using a mouse model of KS (*Kmt2d*$^{+/\beta Geo}$), in which hippocampal memory deficits are ameliorated following treatment with the histone deacetylase inhibitor (HDACi), AR-42. These outcomes are associated with correction of histone H3 lysine 4 trimethylation in the brain. Here, we investigate the effect of both *Kmt2d*$^{+/\beta Geo}$ genotype and AR-42 treatment on DNAm in peripheral blood and neuroanatomy. While peripheral blood may not be considered a "primary tissue" with respect to understanding the pathophysiology of neurodevelopmental disorders, it has the potential to serve as an accessible biomarker of disease- and treatment-related changes in the brain.

Methods: *Kmt2d*$^{+/\beta Geo}$ and wildtype (WT) mice were treated with either 14 days of AR-42 or vehicle, and sacrificed after treatment. Fixed brain samples were imaged using MRI to calculate regional volumes (Lerch *et al.* Methods Mol. Biol., 2011). Blood was assayed for genome-wide DNAm at over 285,000 CpG sites using the Illumina Infinium Mouse Methylation array. DNAm patterns and brain volumes were analyzed in the four groups of animals: WT vehicle, WT treated, *Kmt2d*$^{+/\beta Geo}$ vehicle and *Kmt2d*$^{+/\beta Geo}$ treated.

Results and Impact: We found a striking 10% decrease in total brain volume in *Kmt2d*$^{+/\beta Geo}$ mice (vehicle) compared to WT vehicle, and also defined a DNAm signature in the blood of these mice, which contained orthologs of genes present in the human KS signature. Treatment with AR-42 rescued DNAm aberrations in a subset of signature sites in *Kmt2d*$^{+/\beta Geo}$ mice, highlighting a unique response to HDACi treatment in KS vs. WT mice. While brain volume changes in response to treatment did not meet statistical significance, they did correlate with blood DNAm. As this treatment impacts both neurological deficits and blood DNAm in mice, future KS clinical trials in humans could be used to assess blood DNAm as an early biomarker of therapeutic efficacy.

SENSITIVITY OF MYELOID LEUKEMIA CELLS TO VITAMIN C IS ENCODED IN THEIR REGULATORY DNA AND CHROMATIN TOPOLOGY

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Preclinical studies have suggested defined molecular mechanisms of vitamin C that can effectively inhibit the growth of cancer cells, including myeloid leukemias, especially in combination with approved drugs. These include loss of ATP, enrichment of H_2O_2 and reactive oxygen species as well as the global activation of DNA and histone demethylases[1]. In a murine acute myeloid leukemia (AML) model expressing *IDH1*^{R132H}, a recurrent gain-of-function mutation that inhibits DNA and histone demethylases, we have previously demonstrated that high-dose vitamin C drives demethylation of regulatory elements. We observed that this vitamin C driven "epigenomic reprogramming" induces a pro-differentiation gene expression program that is able to overcome the block of cellular maturation that is characteristic for AML[2].
Clinically, vitamin C has a longstanding history of controversy with some trials in terminal stage cancer patients supporting and others disputing anti-cancer activity. In line with this controversy, we observed that cells within a heterogenous pool of HoxA9/*IDH1*^{R132H} expressing murine bone marrow models respond differently to vitamin C despite overexpressing the same oncogenes. To identify characteristics that associate with response to vitamin C, we have established single-cell-derived AML models that show a binary and reproducible tolerance (n=3) or sensitivity (n=3) to vitamin C. From each model we have generated RNA-sequencing-, DNA (hydroxy)methylation sequencing, and functional data (proliferation, colony-forming-, and *in vivo* engraftment assays) before and after vitamin C treatment. We observed that vitamin C sensitive and tolerant cell lines cluster based on their methylation patterns at regulatory elements, suggesting that distinct epigenetic signatures could be associated with vitamin C sensitivity. Next, we plan to validate these signatures in data from AML patients and cell lines to establish whether methylation fingerprints could predict if patients can benefit from vitamin C as a potent, safe, and cost-effective adjuvant to existing AML therapy regimens, and thus could help to better personalize and to detoxify current therapeutic approaches. Moreover, uncovering epigenetic roadblocks that indicate whether a pool of leukemic cells is able to activate transcriptional programs that correspond with drug tolerance will likely advance our general understanding of drug sensitivity.

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GENOME GRAPHS DETECT HUMAN POLYMORPHISMS IN ACTIVE EPIGENOMIC STATES DURING INFLUENZA INFECTION

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Background Epigenomic experiments can be used to survey the chromatin state of the human genome and find functionally relevant sequences in given cells. However, the reference genome that is typically used to interpret these data does not account for SNPs, indels, and other structural variants present in the individual being profiled. Fortunately, population studies and whole genome sequencing can assemble tens of thousands of sequences that are not in the reference, including mobile element insertions (MEIs), which are known to influence the epigenome. We hypothesized that the use of a genome graph, which can capture this genetic diversity, could help identify more peaks and reveal notable regulatory sequences hidden by the use of a biased reference.
Results Given the contributions of MEIs to the evolution of human innate immunity, we wanted to test this hypothesis in macrophages derived from 35 individuals of African and European ancestry before and after in-vitro *Influenza* infection. We used local assembly to resolve non-reference MEIs based on linked reads obtained from these individuals and reconstructed over five thousand Alu, over three hundred L1, and tens of SVA and ERV insertions. Next, we built a genome graph representing SNPs, indels and MEIs in these genomes and demonstrated improved read mapping sensitivity and specificity. Aligning H3K27ac and H3K4me1 ChIP-seq and ATAC-seq data on this genome graph revealed between 2 to 6 thousand novel peaks per sample. Notably, we observed hundreds of polymorphic MEIs that were marked by active histone modifications or accessible chromatin, of which 12 were associated with differential gene expression. Lastly, we found a MEI polymorphism in an active epigenomic state that is associated with the expression of TRIM25, a gene that restricts influenza RNA synthesis.
Conclusion Our results demonstrate that the use of graph genomes capturing genetic variability can reveal notable regulatory regions that would have been missed by standard analytical approaches.

THE *MEST* DMR REGULATES *KLF14* IMPRINTING VIA ALLELE-SPECIFIC SUB-TAD STRUCTURES

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The imprinted gene *Mest* is regulated by a gametic differentially-methylated region (gDMR) at its promoter CpG island (CGI), retaining oocyte-derived DNA methylation (DNAme) throughout development. Consequently, *Mest* is a paternally-expressed gene. The maternally-expressed gene *Klf14* is located ~200 kb downstream of *Mest* on mouse chromosome 6. Although its promoter CGI is kept unmethylated in most tissues, maternally-inherited DNAme is paradoxically required for *Klf14* expression. Although imprinting at *Mest* is well understood, the mechanism regulating *Klf14* imprinting is still unknown.
Here, we show that *Mest* and *Klf14* reside within the same topologically associating domain (TAD) in mouse embryonic stem cells (ESCs), defined by sites of biallelic CTCF binding at the boundaries. To study imprinting aspects in cell-based assays, we established a new panel of F1 hybrid ESC lines from reciprocal crosses between C57BL/6J and CAST mice. Upon differentiation of these ESCs into FLK1+ vascular progenitor cells, we confirmed maternal allele expression of *Klf14.* Using allele-specific 4C-seq in our undifferentiated F1 hybrid ESCs, we show that CTCF binding to the unmethylated *Mest* gDMR generates a paternal allele-specific sub-TAD required for *Klf14* silencing. Using CRISPR-Cas9, we deleted part of the *Mest* gDMR containing this CTCF binding site and obtained F1 ESCs with paternal, maternal, or homozygous deletions. Whereas deletion of the DNA methylated maternal allele has no effect, removing the CTCF binding from the unmethylated paternal allele leads to biallelic *Klf14* expression in FLK1+ cells and loss of the paternal sub-TAD in ESCs.
These results provide a mechanistic model for the imprinting of *Klf14* and establish the *Mest-Copg2-Klf14* locus as a new imprinting cluster regulated by a single imprinting control region (ICR), the *Mest* gDMR. Our observations also define a new role for maternally methylated gDMRs, which are all associated with promoter-proximal functions: our results show they can also exert long-range effects via allele-specific modulation of TAD structures.

THE REGULATION OF THE NEGATIVE ELONGATION FACTOR (NELF) COMPLEX IN MUSCLE REGENERATION

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Muscle regeneration functions through activated satellite cells repairing damaged muscle tissue. This requires quick responses to environmental stimuli to enable the necessary cell state changes. RNA Pol II promoter-proximal pausing is a key regulator of rapid response genes and is stabilized by binding of the negative elongation factor (NELF) complex. Recent studies found that despite NELF targeting 7000 genes in proliferating muscle cells, a loss of functional NELF results in the dysregulation of only a small subset of target genes. This raises the question of how NELF is selectively regulated at various subsets of genes in muscle regeneration. It is our hypothesis that NELF responds to distinct cellular signalling pathways at different subsets of genes to allow for the selective regulation of target gene expression. RNAseq was performed using a mouse model with a satellite cell specific conditional knockout of NELF-B at three timepoints following injury, with analysis identifying subsets of NELF target genes with differential gene expression. Furthermore, we compared the expression of NELF target genes through regeneration and identified subsets of genes with significant changes in expression at specific timepoints. Using motif analysis of the regulatory sequences of the gene subsets, we identified transcription factors potentially interacting with NELF to regulate target gene expression in a signal-dependent manner. Currently, we are exploring candidate factors and their interactions with NELF in myoblasts to understand the regulation of muscle regeneration in response to injury.

CHANGES IN PMD METHYLATION DURING THYMOCYTE DEVELOPMENT

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Mammalian cells from different tissues or even within the same tissue fulfill unique roles, while sharing the exact same genome. To allow these characteristic functions, gene expression needs to be regulated at several levels, one important aspect of this being DNA methylation (DNA-meth), a key epigenetic mechanism. On a genome wide level, DNA-meth indicates relative accessibility of genes in the chromosomal landscape. There, regions are bioinformatically differentiated based on their methylation level. The heterochromatin characteristically consists of partially methylated domains (PMDs).
In previous studies of human peripheral CD4+ T cells, global DNA-meth, especially in PMDs, was reduced following T cell differentiation and proliferation (Durek et al., Immunity 2016). Naïve T cells in the periphery already went through differentiation and proliferation during their maturation in the thymus, while maintaining high DNA-meth. Analyzing the changes in DNA-meth during this thymic development could give insights into cause and consequence for the loss of methylation in PMDs.
For this, I isolated murine thymocyte subpopulations using reported cell lineage markers FACS markers. Preliminary analysis of their DNA-meth revealed an unexpected gain of methylation, indicating contaminating populations. Using a cell atlas of human thymic development as a reference (Park et al., Science 2020), I further characterized the composition of cells in pediatric human thymi utilizing FACS with a 21-color parameter panel. I identified the contaminations in the DN1 and DN2 population as NK cells, ILC1, ILC3, NKT progenitors and pDCs. While B cells and cDCs were not present in sorted subsets, I could identify these populations in other, unsorted thymic populations. Following these characterizations, I am now able to efficiently sort pure thymocyte subpopulations.
Next steps include DNA-meth analysis of those pure pediatric T cell progenitor populations to highlight differentially methylated regions and genome wide effects. Furthermore, in collaboration

with a group in Jena, I will analyze T cell progenitor DNA-meth in a humanized mouse model, assessing differences between this model, the murine development, and the human development.
Ultimately, this research can improve our understanding of thymic development and maintenance of DNA-meth. Further, this might help counteracting possible side effects of loss of DNA-meth during adaptive T cell therapy, improving laboratory protocols and therapy.

DNA REPLICATION SPEED REGULATES HETEROCHROMATIC DNA-METHYLATION LOSS DURING CELLULAR AGING

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It is well known that the efficiency of the immune system declines with age ("immune aging"), leading to impaired vaccination responses and increased susceptibility to infections. One important contributor to immune aging is the progressive proliferation and differentiation of T-lymphocytes. Long-lived T-cells undergo repetitive rounds of activation-induced or homeostasis-driven proliferation over the lifetime of a human being. Therefore, the activation and proliferation history of T-cells, rather than the passing of chronological time, determines aging of the immune system.
In a genome-wide analysis of DNA methylation in primary human T-cell subsets, we discovered a progressive, heterochromatin-restricted loss of DNA methylation, which is correlated to the proliferation history of differentiated T cells. These regions were reported earlier as partially methylated domains (PMDs), which show proliferation-associated hypomethylation also in other somatic cell types and in cancer. However, the molecular mechanism of PMD methylation loss and its functional consequences have not been delineated so far.
In our study, we show for the first time that PMD hypomethylation in in vitro primary human T-cell cultures is regulated by DNA replication speed during S phase. These results complement earlier computational studies, which have shown a correlation between replication timing and PMD methylation levels. In upcoming over-expression and knock-out approaches, we want to verify our hypothesis, that PMD hypomethylation occurs due to a shortened time window of methylation maintenance during DNA replication rather than an enzyme-driven demethylation process. Furthermore, our preliminary data indicate that PMD hypomethylation correlates with a greater differentiation potential. Follow up experiments are ongoing to clarify the link between the proliferation history of long-lived T-cells and their increased potential to undergo further differentiation steps.

SEX-SPECIFIC DNA METHYLATIONS IN PLACENTAL CELLS

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The placenta is a core organ that mediates fetal growth and development. It consists of distinct cell types derived from both the trophectoderm and the inner cell mass from a blastocyst. Placental sex chromosome complement (XX or XY), which is typically the same as that of the fetus, may influence both structural and functional aspects of the placenta and risk for various pregnancy complications. DNA methylation (DNAm) is an epigenetic mark that can regulate gene expression and may be used as a biomarker for placental health. However, the characterization of human placental DNAm based on the interaction of both placental sex and cell type is poorly understood.
To better characterize sex-influenced DNAm in placental cell types, we measured genome-wide DNAm using the Illumina EPIC DNA methylation array for cell-sorted samples from 10 XX (female) and 9 XY (male) term placentas. Cell types examined included trophoblasts, endothelial, stromal and Hofbauer cells, as well as bulk chorionic villi. Using linear modelling with statistical cutoffs of $|\beta\text{-value}| > 0.05$ and FDR < 0.05, we identified 39 autosomal sex-influenced CpGs in endothelial cells, while less than 5 CpGs were identified in other cell types. For the sex chromosomes, we used stricter statistical cutoffs of $|\beta\text{-value}| > 0.1$ and FDR < 0.05 and identified more than 1,000 cell type-influenced CpGs that differed in DNAm between endothelial, stromal and Hofbauer cells versus chorionic villi on the X chromosomes of both sexes. We identified more than 100 cell-influenced CpGs in endothelial, stromal and Hofbauer cells on the Y chromosome. Using the total and significant DMCs from X and Y, we analyzed the frequency of CpG island regions to understand the distribution of represented CpG locations. We found that the distribution of DNAme on the X of XX from endothelial and stromal cells presented a similar distribution as the single X of XY cells, with little methylation of CpG islands. However, DNAme on the X from XX cells was higher in island region and lower in open sea regions compared to the single X from XY cells. This increased methylation of gene promoters is expected as a result of X-chromosome inactivation. The distributions of Y DNAme were similar except for the Hofbauer cells, and N_shore enrichment seemed to be twice as high as the X from XX and XY cells.
Our study provides a characterization of DNAm sex differences in autosomes and XY chromosomes of placental cell types and emphasizes the role endothelial cells may contribute to these sex differences, as well as cell-specific differences in the Y chromosome, which is often neglected in such analyses. This work will help identify mechanisms underlying sex differences associated with placental complications and pregnancy outcomes.

CELL-TYPE-SPECIFIC GENETIC-TO-EPIGENETIC RELATIONSHIPS IN THE HUMAN BREAST

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Understanding the interaction between genetic and epigenetic states is fundamental to the study of development and mechanisms of disease. Studies that have explored these relationships have largely leveraged population-scale genotype surveys to associate genetic polymorphisms with epigenetic states in whole blood or other heterogenous tissue types. However, epigenetic states are cell-type-specific, raising the possibility of cell-type-specific genetic-to-epigenetic relationships that could drive specific functional states and disease predisposition. To address this question, I analyzed histone-modification-enriched regions for six distinct histone marks across four functionally distinct human breast cell types from eight phenotypically normal women to identify germline variants that influence cell-type-specific epigenetic states. I define the incidence by cell type and histone mark of these interactions across breast cell types, terming them *cis* histone binary trait loci, or *cis*-hBTLs. These *cis*-hBTLs were found to be globally enriched in disruption/creation of transcription factor (TF) motifs, and *cis*-hBTLs found in specific cell types were more strongly enriched for TF motif disruption/creation for TFs upregulated in the same cell type based on previously published *in silico* and *in vitro* models of TF binding. Active histone marks and intergenic regions were also enriched in *cis*-hBTLs. Nine *cis*-hBTLs were prioritized based on strong correlation between variant allele and nearest gene expression, and these were found to be enriched in genes and long noncoding RNAs implicated in breast carcinogenesis. A subset of *cis*-hBTLs was then validated through epithelial breast cell lines, which reproduced the association between variant allele and linked gene expression. My analysis suggests that genotype is variably interpreted across functionally distinct breast cell subtypes and that this variation may have implications for understanding genetic influences on *cis*-regulatory elements and disease.

NATURAL SELECTION AND CHROMATIN REMODELING HAVE SHAPED INTERSPECIES DIFFERENCES OF IMMUNE RESPONSE ACROSS PRIMATES.

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Humans are more prone to inflammatory and infectious diseases than other primate species such as macaques and baboons. These differences, at least partially, are expected to result from interspecies differences of immune response. How the innate immune response from these species is functioning and diverges across different immune cell populations remains poorly understood. Moreover, the regulatory elements that dictate the response across these species have not been yet characterized. Herein, we characterized immune response and its regulatory landscape from human, rhesus macaque, olive baboon and one basal primate, ring-tailed lemur upon stimulation with lipopolysaccharide (LPS) at single cell resolution. We found that immune response strength is correlated with the primate phylogeny, the highest in human and the lowest in lemur with monocytes being the most responsive immune cells across species. The regulatory landscape was found to change significantly only in simian primates after stimulation. Transcription factor (TF) footprinting analysis identified significant signal in stimulated cells for a number of inflammatory TFs. Those TF footprints were found to be significantly enriched closer to genes with the highest response in humans. Further analysis of motifs within the inflammatory TF footprints identified a signal of adaptive evolution highlighting the role of natural selection and chromatin remodeling in dictating the evolution of immune response in primates.

THE ADAPTED ACTIVITY-BY-CONTACT MODEL FOR ENHANCER-GENE ASSIGNMENT AND ITS APPLICATION TO SINGLE-CELL DATA

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Identifying regulatory regions in the genome is of great interest for understanding the epigenomic landscape in cells. One challenge in this context is to find the target genes whose expression the regulatory regions affect. A recent successful method is the Activity-By-Contact (ABC) model [1], which scores enhancer-gene interactions based on enhancer activity and the contact of an enhancer to its target gene. It requires two types of assays to measure enhancer activity, which limits the applicability. Moreover, there is no implementation available which would allow for an integration with transcription factor (TF) binding information or an efficient analysis of single-cell data.
We show that the ABC-model is comparably accurate with only one assay to measure enhancer activity. Further, we demonstrate that it can yield a higher accuracy by adapting the enhancer activity according to the number of contacts the enhancer has to its candidate target genes. We combined our adapted ABC-model with TF binding information and illustrate an analysis of a public single-cell data set of the human heart [2]. Leveraging snATAC-seq and bulk H3K27ac HiChIP data [3], we predicted regulatory interactions for eight defined cell types. Based on these enhancer-gene interactions, we constructed cell type-specific gene-TF affinity matrices, which represent the potential regulatory impact of TFs on a gene. As an example downstream application of the gene-TF matrices, we trained a gene expression prediction model, which allows to prioritise candidate TFs that drive cell type-specific expression.

All executed processing steps are incorporated into our new computational pipeline STARE, which is available at https://github.com/schulzlab/STARE.

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

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Epigenetic modifications alter the properties of DNA and chromatin, which are crucial for proper control of gene regulation during normal mammalian development. Methyl groups added to Histone H3 tail on lysines at position 27 and 36 have been shown to have an interesting antagonistic interplay, which specifically affect DNA methylation, regulation of cis-regulatory elements (CRE) and gene expression, in intergenic regions. Alteration of that specific interplay is involved in multiple malignancies including pediatric brain tumors, head and neck cancers, chondroblastoma, multiple myeloma and pediatric acute lymphoblastic leukemia. Moreover, mutations in the enzymes responsible for the deposition of methyl groups on H3K27 (PRC2), H3K36 (NSD1) and DNA (DNMT3a) are causing different yet phenotypically similar childhood overgrowth syndromes, respectively: Weaver Syndrome, Sotos Syndrome and Tatton-Brown-Rahman syndrome.
NSD1 and NSD2 are both H3K36 methyltransferase enzymes, sharing similar catalytic chromatin binding domains. Yet, phenotypic outcomes of mutations in those two genes appear to differ. For example, loss of function mutations in NSD1 (but not in NSD2) are found in a subset of head and neck tumors, while activating mutations leading to NSD2 overactivity are found in multiple myeloma and pediatric acute lymphoblastic leukemia. Moreover, alterations of NSD2 is associated with Wolf-Hirschhorn syndrome, characterized by growth retardation, compared to Sotos Syndrome (alteration of NSD1) which features overgrowth features.
Very little is known about the functional differences between NSD1 and NSD2. To answer this question, we used patient derived cell lines of HNSCC carrying wild type for NSD1 and created CRISPR isogenics for NSD1 and/or NSD2 to evaluate the levels of H3K36me2 and H3K27me3 genome wide (mass spec) or at specific genomic locations e.g. genic vs intergenic, or promoter (ChIPseq analysis). So far, we find that knock out of NSD1 has a dominant effect on H3K36me2 levels in intergenic regions because additional knock out of NSD2 doesn't confer an additional reduction of that mark. Intriguingly, knocking out NSD2 by itself leads to both decrease and increase levels in H3K36me2 in different regions of the genome. We are in the process of determining how NSD2 acts and where on the genome compared to NSD1. We also wish to contrast these observations with the effect of the dominant H3K36M overexpressing lines we produced, which show a complete depletion of all H3K36 methyl marks in all intergenic regions. Finally, we wish to evaluate the correlation that NSD1-KO, NSD2-KO or H3K36M have on general gene expression, and if NSD2-KO and H3K36M also affect CRE elements in intergenic regions as we previously observed in NSD1-KO.

ZIC2 AND ZIC3 ARE INTEGRAL FOR SPECIFICATION OF THE POST-IMPLANTATION EPIBLAST-LIKE TRANSCRIPTIONAL PROGRAM

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Embryonic development entails coordinated changes in regulatory circuits driven by strict epigenetic mechanisms. The epiblast lineage is subject to dynamic transcription factor activity as it progresses through peri-implantation. Using a multi-omic approach, we find that ZIC2 and ZIC3 are integral during this transitory phase to establish primed pluripotency in humans. In primed hESCs, ZIC2 and ZIC3 have largely overlapping roles. They act together to open chromatin and activate genes that promote progression towards the primed epiblast and ensure Polycomb-mediated represson of genes associated with differentiation to extra-embryonic and post-gastrulation lineages. We identify SOX2, BRG1 and CHD4 as potential co-factors of ZIC2/3, indicating a mechanistic basis for how *ZIC* factors can underpin two contrasting facets of gene regulation in the same cell type. Concomitant depletion of ZIC2/3 results in loss of primed hESC identity and reduced viability. Interestingly, we observe de-repression of Hominoid-specific LTR5Hs upon ZIC2/3 loss, which plays a prominent role in defining the enhancer landscape of pre-implantation embryos. Overall, these two divergent axes of regulation are essential for specification towards the post-implantation epiblast lineage in humans and sheds light on how conserved transcription factors can gain a species-specific role in human embryogenesis.

ASSESSING THE QUALITY OF SEX AND GENDER ANALYSIS AND DISCOURSE IN THE CANCER EPIGENETICS FIELD

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Although sex and gender considerations have been touted to be vitally important to epigenetic expression and regulation in cancer research, little has been done to assess the quality of sex and gender-based analyses (SGBA) and the kinds of discourse within this field. We systematically undertook a scoping review according to the PRISMA-ScR guidelines to analyze multiple facets of sex and gender-based analysis and discourse in the cancer epigenetics field from 2010 onwards. Peer-reviewed scientific literature was searched in PubMed, Google Scholar and Scopus with MeSH terms and synonyms for the keywords: cancer, epigenetics, sex, and gender. Two independent reviewers performed the data screening, extraction, and quality appraisal process. A total of 185 studies were included, with 111 studies comprising 5 cancers with the most prevalent cancer publications within the epigenetics field. Types of eligible studies include basic research, epidemiological, clinical, and reviews. In the first part of the study, the full text and supplemental material of all eligible studies from the top 5 cancers (colorectal, gastric, head and neck, hepatocellular carcinoma, lung) were analyzed and codified by a team of epidemiologists and bioethicists to determine the quality of sex/gender analysis and discourse.
This presentation will describe our results, including studies that explicitly stated sex and gender analysis to be their primary aim, studies that performed detailed sex or gender analyses, and studies that only stratified results by sex/gender. Interestingly, although sex and gender were a key facet in all the eligible studies, few provided an explicit well-defined and working definition of the terms 'sex' or 'gender' based on the international definition provided by the WHO, and many studies conflated the concepts and usage of the 'sex' and 'gender' terms. No studies included considerations of LGBTQIA+ or sex and gender minorities in any part of the text. Further, we will report the primary sex/gender-based findings and epigenetic effect by sex. A critical appraisal was also performed for each study according to SAGER guidelines to assess the quality of sex or gender analysis. The resultant limitations that were found will be discussed, such as studies which statistically analyzed sex but failed to account for co-variates, studies which lacked statistical power in their generalizations, studies with small sample populations ($n < 100$) or imbalances in the proportion of males versus females. Recommendations will be made to improve the quality and accuracy of reporting and analyzing sex/gender data. For example, we noticed that most studies fail to mention gender differences when attributing a cause of the purported sex differences. Future directions include investigating the quality of sex/gender analysis for all cancers (185 total studies) and performing a meta-analysis to assess the accuracy of reported sex trends in specific cancer types.

TRANSPOSABLE ELEMENTS REGULATE CELL FUNCTION AND DIFFERENTIATION THROUGH CELL TYPE SPECIFIC EPIGENOME ASSOCIATIONS

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Transposable elements (TEs) are DNA elements able to copy themselves within the genome. This ability has led to interest in their potential as regulatory elements that spread within the human genome throughout evolution. Despite minimal TEs expression due to silencing, TEs still manage to have a noticeable impact on host genome in numerous contexts. For instance, some TEs have been shown to behave like cis-regulatory elements, enhancers and even to have been co-opted by the host genome. This highlights that a large part of TEs impact might come from their relationship with the epigenome rather than their expression. However, a comprehensive analysis that relates multiple TEs with all the main histone marks across distinct cell types remains lacking. Here we leverage a comprehensive dataset of 3398 histone mark ChIP-seq samples across tissues from the International Human Epigenome Consortium (IHEC) reprocessed data and examine the relationship between TEs, the epigenome and cell types. We show that TEs have drastically different enrichments levels in different histone marks and the enrichment further varies between cell types. We find that TEs are generally depleted but that L1 is enriched almost exclusively near H3k9me3 histone modification, while MIRs were highly enriched in H3K4me1, H3K27ac and H3K27me3 and Alus were enriched in H3K36me3. Furthermore, we present a generalised profile of the relationship between TE enrichment and TE age which reveals a few TE families (Alu, MIR, L2) as potential genome regulators. We also find some significant differences in TE enrichment between cell types and that TE enrichment can be cell type specific in certain contexts. These results further support existing research while highlighting novel associations and providing a comprehensive profile of TEs across cell types and histone marks.

THE ROLE OF ARID1B DURING EXCITATORY AND INHIBITORY NEURONAL DIFFERENTIATION

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The process of embryonic stem (ES) cell differentiation into post-mitotic cell types is often associated with epigenetic regulation such as chromatin remodeling. One of the key chromatin remodeling complexes is the BAF complex, which has cell lineage-specific subunits. Arid1b is a subunit found in neural progenitor and neuron-specific BAF complexes. Mutations of Arid1b have been linked to neurological disorders, often causing intellectual disability and impaired motor skills. Mutations of Arid1b in mice affect the balance of excitatory and inhibitory neuronal signals leading to abnormal cognitive behaviors and low motor skills. What remains unknown is the genomic mechanism by which Arid1b regulates gene expression within excitatory and inhibitory neurons. Using mouse ES cell-derived neurons, we show that ES cells containing the Arid1b knockout impair proper differentiation into excitatory motor neurons and inhibitory GABAergic neurons by inhibiting neuronal gene expression and dendritic growth. Arid1b knockout cells remain in cell cycle in the later stage of differentiated cells compared to wildtype, implying the role of Arid1b in cell cycle exit. Furthermore, we reveal genome-wide changes in active enhancer genomic regions, enriched by histone 3 lysine 27 acetylation, upon Arid1b knockout. Together, we show that Arid1b is required for the differentiation of ES cells into postmitotic motor neurons and GABAergic neurons. Understanding the role of Arid1b in neuronal

differentiation is crucial to better understand the causative factors of the neurological disorders linked to Arid1b mutations.

DISSECTING THE ROLES OF H3K27ME3 AND H2AK119UB1 IN TRANSCRIPTIONAL SILENCING FOLLOWING NSD1 DEPLETION

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Sotos Syndrome is a neurodevelopmental disorder caused by heterozygous loss-of-function mutations in *NSD1*, an enzyme responsible for the deposition of two methyl groups on lysine 36 of histone H3 (H3K36me2). A recent study revealed aberrant gene expression in blood samples from Sotos Syndrome subjects, with the majority of affected genes downregulated. Currently, the molecular mechanism by which loss of NSD1 leads to altered gene expression remains unclear. Possibly, the gain in PRC2-mediated H3K27me3, a transcriptionally repressive chromatin mark, that occurs following NSD1 depletion is involved. PRC1-mediated mono-ubiquitination of H2AK119 (H2Aub) may also play a role, as H2Aub appears to be more important for transcriptional repression than H3K27me3, but if its deposition is altered in the absence of NSD1 is unknown. To determine whether altered H3K27me3 and/or H2Aub deposition are responsible for aberrant gene expression in the absence of NSD1, we employ an *in vitro* system that mimics early development, namely naïve mouse embryonic stem cells (nESCs) differentiated into epiblast-like cells (EpiLCs). Using RNAseq, we observe altered gene expression in *Nsd1* knockout (KO) nESCs and EpiLCs, coincident with an increase in global H3K27me3, but not H2Aub, as determined by Western blot. Analysis of the distribution of H3K27me3 and H2Aub by ChIPseq is ongoing. *Nsd1* KO EpiLCs show the largest transcriptional dysregulation, with the majority of genes downregulated. We find that 60% of genes downregulated and 45% of genes upregulated in *Nsd1* KO EpiLCs are normally induced and repressed, respectively, during differentiation, including so-called 'hallmarks' of differentiation. These data indicate that *Nsd1* KO leads to a defect in EpiLC differentiation. To determine if a reduction in H3K27me3 can alleviate the lack of gene induction observed in *Nsd1* KO cells, we treated cells with the PRC2 inhibitor Tazemetostat. Reduced H3K27me3 was confirmed by Western blot and RNAseq will be performed on these cells. In addition, to confirm our findings from the inhibitor treatment and address if H2Aub plays a role in the observed transcriptional repression, we will perform RNAseq on *Nsd1;PRC2* and *Nsd1;PRC1* double KO cells. We anticipate that the insights gained from this study will contribute to a better understanding of the molecular mechanism underlying the transcriptional alterations observed in Sotos Syndrome subjects.

TYPE B ULTRA LONG-RANGE INTERACTIONS IN PFAS (TULIPS) ARE RECURRENT EPIGENOMIC FEATURES OF PFA EPENDYMOMA

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Posterior Fossa Group A (PFA) ependymomas are pediatric brain tumors with extremely poor survival outcomes. As protein-coding mutations in PFA are exceedingly rare, the underlying etiology of these tumors remains elusive. Elevated CpG island methylation and depletion of H3K27me3 have been described in PFA, leading to the hypothesis that PFA may be driven by a dysregulated epigenetic state. In this study, we sought to determine how three-dimensional (3D) genome features (such as DNA loops, domains,

and compartments) differ between pediatric brain tumors. We performed Hi-C sequencing on a collection of 64 patient specimens and patient-derived primary cultures that collectively span multiple subgroups of ependymoma, medulloblastoma, high-grade glioma, and non-neoplastic brain. For certain samples, we further performed RNA-seq, histone modification ChIP-seq, or whole-genome bisulfite sequencing to allow multiomic data integration. Overall, the 3D genome organization of PFA samples appeared distinct from other tumor types. We identified and defined TULIPs: a subset of type B compartments, separated by genomic distances greater than 10 Mbp, that exhibit a striking fivefold increase in reciprocal interaction strength. These TULIPs recurred at the same genomic positions across the vast majority of PFA samples with minimal representation among other tumor or non-tumor samples. TULIPs displayed enrichment for heterochromatic features such as H3K9me3 and late replication timing and were depleted of euchromatic features such as H3K27ac and protein-coding genes. By using immuno-fluorescence for H3K9me3 and oligo-FISH to label TULIP regions, we demonstrated that TULIP regions are more compact in PFA than other tumors. Finally, by applying inhibitors of H3K9 lysine methylation to PFA cultures we showed that TULIPs become more diffuse and cell viability is reduced. Altogether, this work defines TULIPs as highly recurrent epigenetic features of PFA tumors.

DNA METHYLATION IS ESSENTIAL FOR THE VIABILITY OF TROPHOBLASTIC STEM CELLS

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DNA methylation is an epigenetic mark deposited by DNA methyltransferases (DNMTs) to regulate the expression of genes, a process that is crucial for appropriate embryonic development. After implantation of the blastocyst, distinct methylation profiles are established in the epiblast and the trophoblast, which generate the embryonic and placental tissues, respectively. The placenta is essential for proper fetal development during pregnancy by orchestrating gas and nutrient exchange and by secreting pregnancy hormones. Murine trophoblastic stem cells lacking in DNA methylation are shown to survive and contribute to the placenta, suggesting that DNA methylation is not essential for survival of murine placental cells. We sought to determine the function of DNA methylation in human placental development by deleting DNMT1 in human trophoblast stem cells (hTSC) using CRISPR/Cas9. Near complete deletion of DNMT1 was observed, but DNMT1 KO cells did not survive long-term in culture and were outcompeted by remaining DNMT1^{+} cells. DNMT1 KO cells showed dramatic upregulation of germline genes and the endogenous retrotransposon HERV-Fc1. Surprisingly, DNMT1 KO hTSCs show cytoplasmic localization of the key trophoblast transcription factor TEAD4. Our preliminary results suggest increased apoptosis of the DNMT1 KO cells. Our results show a striking species-specific difference in which DNA methylation is critical for human trophoblast cellular homeostasis.

DISCERNING THE REGULATORY PROCESSES UNDERLYING CELL STATES IN LOCALLY ADVANCED METASTATIC PROSTATE CANCER USING SINGLE-CELL MULTIOMICS

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Prostate cancer (PCa) is one of the most common and most heterogeneous cancers in biological males. One in six patients present with locally advanced disease and have tumour dissemination in their pelvic lymph nodes (LN) upon diagnosis. Presence of LN-disseminated tumours is an important prognostic factor for poor patient outcome. However, the underlying biological pathways supporting aggressive LN-disseminated PCa remains an open question. For this reason, profiled chromatin accessibility and gene expression at the single-cell resolution from 38 samples, originating from multiple matched primary localized tumours and LN tumours in 5 patients. We present data from a total of ~300,000 high-quality cells and demonstrate significant heterogeneity between patients in malignant epithelial cells, but not stromal cells. We show that PCa cells of the prostate-localized tumours versus LN tumours vary across a 'developmental-injury' axis, reflected in their gene expression and chromatin accessibility profiles. We attempt to discern the regulatory elements supporting these phenotypes and reveal the TF motifs likely governing this axis. Collectively, our dataset and analysis provide a detailed resource that links transcriptional and chromatin states to the underlying cell state heterogeneity in locally advanced PCa. Thereby, enabling us to identify biological processes to guide the development of precision medicine in aggressive cancer phenotypes.

GENOME-WIDE CHANGES IN CHROMATIN ACCESSIBILITY AND BINDING FACTORS IN THE PREGNANT AND LABOURING MYOMETRIUM

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At labour, the myometrium transitions from quiescent to contractile and this switch is thought to be controlled by changes in gene expression. Despite these gene expression changes, our recent work has shown that there was little to no associated increase in the active histone modifications between pregnancy and labour. However, we have recently applied ATAC-seq in the pregnant, labouring, and postpartum mouse myometrium which displays dynamic changes in open chromatin that reveals shifts in the regulatory TFs that bind them. Comparison between timepoints revealed that approximately 38% of all peaks are shared in the myometrium

across all timepoints. Though there are many shared accessible chromatin regions across all timepoints, there were several timepoint-specific regions. Further analysis revealed that approximately 13% of peaks are specific to pregnancy and 21% are specific to labour. Interestingly, the most dramatic change in chromatin accessibility occurred between Day 17 and TNIL samples, with an increase in 1074 regions at TNIL compared to D17 and only 18 regions that are differentially accessible at D17 in comparison to TNIL. To identify any specific proteins that may drive changes in chromatin accessibility, the sequence at accessible regions was evaluated for motif enrichment. Differential motif enrichment analysis revealed an enrichment for progesterone receptor (PR) motifs at the D17 stage whereas several motifs for factors belonging to the activator protein-1 (AP-1) family were highly enriched at the TNIL and LAB stages. The analysis also shows differential motif enrichment at each timepoint, suggesting changes in the TFs that regulate chromatin accessibility and gene expression changes, and thus the switch to contractility in the myometrium. We observed an overrepresentation of the AP-1 factors at the TNIL and LAB stages, suggesting a genome-wide role for the AP-1 family in controlling the gene expression changes that are associated with contractility and the timing of labour onset. Together, these data suggest that although there are no changes observed in the histone acetylation and methylation profiles, there are dynamic changes in the accessible chromatin landscape, coupled with the TFs that bind them, which underpin the quiescent-to-contractile switch in myometrium.

MICROGLIA AS A MEDIATOR OF THE 'TWO-HIT' HYPOTHESIS

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An emerging body of evidence reveals that infections during the perinatal period can contribute to marked alterations in cognitive and affective behaviors later in life and increased risk for various neuropsychiatric and neurodevelopmental disorders including Schizophrenia and Autism Spectrum Disorder (ASD). Several clinical and postmortem studies of these disorders found increased activation of microglia, the brain's resident immune cells. Microglia are uniquely long-lived cells that self-renew over one's lifespan, suggesting that disruptions in early microglial development can have long-lasting impacts on cellular function and disease development. In addition to responding to injury and infection, microglia also play critical roles in normal brain function, interacting with other brain cell types to regulate neuronal cell number, shape brain circuitry, and fine-tune neuronal connections. Adolescence is a sensitive stage in human development marked by physical, cognitive, and social changes that are supported by significant functional reorganization and maturation of underlying neural circuitry in the brain. These changes make adolescence an especially vulnerable period when disruptions in microglial function can disproportionately impact long-term brain function. We hypothesize that a perinatal immune activating event will epigenetically reprogram microglia to hyper-respond to a secondary immune challenge during adolescence, and ultimately have prolonged effects on brain function and behaviour. Previous studies have shown that peripheral inflammation, which can be experimentally induced through low-dose lipopolysaccharide (LPS) injections, triggers immune responses in the brain that primarily occur through microglia activation. In this study, we model early life infection by administering LPS challenges on postnatal days 4 and 6 (P4&6), which we have demonstrated produces exaggerated pro-inflammatory cytokine gene expression (*Il1b*, *Tnfa*) after a second LPS challenge during adolescence (P30) in rats. We are currently characterizing DNA methylation changes that may shape enhanced adolescent microglial gene expression in response to early postnatal inflammation. Disentangling how early life infections shape sensitivity to immune challenges later in life will be invaluable to clarifying the commonly reported links between neuroinflammation and brain disorders.

ACTIVATION OF THE MIRNA CLUSTER C19MC CONFERS DIFFERENTIATION POTENTIAL INTO TROPHOBLAST LINEAGES UPON HUMAN PLURIPOTENT STEM CELLS

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The first cell fate decision in mammals occurs when totipotent blastomeres differentiate into either the inner cell mass (ICM) or trophectoderm (TE). The ICM further differentiates into the epiblast and primitive endoderm. The epiblast gives rise to the entire fetus, the primitive endoderm contributes to the yolk sac, and the TE generates the placenta. Embryonic stem (ES) and trophoblast stem (TS) cells have been derived from ICM and TE cells, respectively.
Recent studies reveal that naïve human ES (hES) cells can spontaneously differentiate into trophoblast stem-like (hTSL) cells. The hTSL cells have similar proliferation and differentiation capacities and transcriptome and methylome profiles to human TS (hTS) cells. Interestingly, there has also been accumulating evidence suggesting that primed hES cells spontaneously differentiate into trophoblast lineage cells upon BMP4 treatment. Many researchers have utilized primed hES cells to study the development and function of the TE lineage, but there are skeptical views on these studies. Primed hES cells are most closely similar to late post-implantation epiblast cells, and post-implantation epiblast cells are unlikely to contribute to the TE lineage. It's also been reported that BMP signaling induces

differentiation of primed hES cells into mesoderm or amnion cells, not trophoblast cells. Therefore, it is still controversial whether primed hES cells can differentiate into trophoblast cells.
In this study, we derived hTSL cells from naïve and primed hES cells, which were designated as hTSL^{naïve} and hTSL^{primed} cells, respectively. We then compared their proliferation and differentiation capacities with those of hTSL cells. We found that although hTSL^{naïve} cells exhibit similar properties to hTS cells, hTSL^{primed} cells have much lower proliferation and differentiation potentials. Transcriptome and methylome analyses reveal that a primate-specific miRNA cluster at chromosome 19 (C19MC) is expressed in hTS and hTSL^{naïve} cells but not in hTSL^{primed} cells. Moreover, genome and epigenome editing using CRISPR/Cas systems demonstrate that C19MC is essential for hTS cell maintenance and C19MC-reactivated primed hES cells can give rise to fully potent hTS cells. Thus, we reveal that C19MC activation in hES cells confers a differentiation potential beyond pluripotency. Our findings are fundamental to understanding the epigenetic regulation of human early development and pluripotency.

DETERMINING THE ROLE OF GREB1L IN TROPHOBLAST CELLS

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By analyzing data from CRISPR/Cas9 screens conducted by the DepMap consortium, we have determined that *Growth Regulation by Estrogen in Breast Cancer 1 Like* (*GREB1L*) is selectively essential for the survival of placental cancers called choriocarcinomas. This suggests a role for GREB1L in placental development. GREB-family proteins have been implicated in glycosylation of nuclear receptors, retinoic acid signaling, and vertebrate renal system development. However, GREB1L's role in the placenta has never been investigated. We generated *GREB1L* knockout (KO) human trophoblast stem cells (hTSCs) using CRISPR/Cas9. *GREB1L* KO hTSCs show no morphological or survival abnormality. However, flow cytometry indicates downregulation of the differentiation marker Integrin-α-1, suggesting impaired propensity for differentiation. Using immunofluorescence staining, hCG ELISA, and RNA-sequencing, we determined that loss of *GREB1L* impairs differentiation to syncytiotrophoblast lineage. Additionally, differentiation into extravillous trophoblasts is impaired in the KO cells as demonstrated by flow cytometry. In summary, our preliminary data indicates a role for GREB1L in facilitating differentiation of placental cells.

CHARACTERIZATION OF HISTONE MODIFICATIONS ASSOCIATED WITH MYOD-BOUND ENHANCERS IN REGENERATING MUSCLE

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Skeletal muscle has long served as an excellent model for investigating stem cells due to its regenerative capabilities. Skeletal myogenesis involves a series of specific cell states starting with muscle stem cells (satellite cells) that are activated in response to injury stimuli or during normal maturation of the muscle. Once activated, satellite cells will commit to the myogenic lineage, expand their population, and transform into myoblasts that differentiate into multinucleated myotubes. Cellular differentiation is controlled by intricate epigenetic machinery. A key part of this machinery are super-enhancers, a large group of neighbouring transcriptional enhancer regions. These regions are delineated by signature histone post-translational modifications (PTMs), and an abundance of transcription factors and cofactors. Histone PTMs, placed by epigenetic enzymes, are critical for maintaining chromatin structure and fostering transcription events. These modifications dictate when genes are available for transcription and modulate the degree of gene expression via enhancer-promoter interactions. Therefore, the fate of satellite cell differentiation into muscle tissue is likely reliant on histone marks found at active, muscle-specific enhancer regions. However, the catalogue of histone marks required for an active enhancer is likely incomplete as, to date, there has not been a discovery-oriented approach to identify PTMs found at enhancer regions. We propose a combination of proximity labelling and mass spectrometry to allow for the identification of histone modifications associated with muscle enhancers. Mass spectrometry is particularly powerful at discovering combinatorial PTMs which are likely important as PTMs can function in tandem. Using biotin ligase fused to MyoD, the master transcriptional regulator of myogenic programing, we targeted MyoD-proximal proteins and the histones surrounding MyoD binding sites and subjected the biotinylated proteins to mass spectrometry. Specific combinations of histone marks have been identified on MyoD-associated nucleosomes. Ongoing work is expected to reveal the full set of epigenetic marks that define muscle-specific active enhancers. It is expected that identification of these specific marks will provide new insight into the proteins that regulate temporal and spatial transcription of key genes disrupted in muscle diseases and will be important for the development of therapeutics for treating myopathies.

TFIID SUPPORTS RNA POLYMERASE II TRANSCRIPTION INDEPENDENTLY OF TBP

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Transcription by RNA Polymerase II (Pol II) is initiated by the hierarchical assembly of the Pre-Initiation Complex onto DNA promoters. Decades of in vitro and yeast research have shown that the TATA-box binding protein (TBP) is essential to Pol II initiation by triggering the binding of other general transcription factors and ensuring proper Pol II loading. Here, we report instead that acute depletion of TBP in mouse embryonic stem cells (mESCs) has no global effect on ongoing Pol II transcription. In contrast, acute TBP depletion severely impairs initiation by RNA Polymerase III. In addition, Pol II transcriptional induction through the heat shock response and retinoic acid differentiation occurs normally in the absence of TBP. Furthermore, we show that the TBP paralog TRF2 binds to gene promoters in TBP-depleted cells, but it does not functionally replace TBP in Pol II transcription. Lastly, we show that the TFIID complex can form and bind to gene promoters with distinct dynamics in TBP-depleted cells and supports Pol II transcription in the absence of TBP.

DISTINCT H3K27ME3 AND H3K36ME2 IN HUMAN TROPHOBLAST AND EMBRYONIC STEM CELLS

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The placenta is an essential organ for fetal life, creating a suitable environment and providing nutrients to the developing embryo. The placenta has unique methylome, different from that of embryonic tissue: there is low global DNA methylation, specific methylation of hundreds of CpG islands, and large partially methylated domains (PMDs). However, the mechanisms that control the establishment of placenta-specific DNA methylation remain poorly understood. Histone 3 Lysine 27 trimethylation (H3K27me3), a heterochromatic mark deposited by Polycomb repressive complex 2 (PRC2), and Histone 3 Lysine 36 dimethylation (H3K36me2) are important epigenetic modifications associated with regulating key developmental genes during embryonic development, with H3K36me2 functioning to both antagonize H3K27me3 and promote DNA methylation. To compare the genome-wide distribution of H3K27me3 and H3K36me2 in early lineages, we performed ChIP-seq in human trophoblast stem cells (hTSCs) and primed human embryonic stem cells (hESCs), which approximately correspond to trophoblast, and post-implantation epiblast respectively. Our results show a strongly distinctive H3K27me3 pattern in primed hESC vs. hTSC. Superficially, primed hESCs show higher enrichment for H3K27me3 at promoters of developmentally regulated genes by ChIP-seq. However, western blotting and mass spec analysis show far higher H3K27me3 in hTSCs, much of it dispersed in large domains that do not appear as discreet peaks in ChIP-seq analysis. Furthermore, while primed hESCs have widespread H3K36me2, hTSCs have interspersed regions of H3K36me2 and H3K27me3, with strong correlation between H3K36me2 and DNA methylation in hTSCs. Lastly, CRISPR-mediated deletion of the key PRC2 component EED resulted in rapid death of hTSCs. Our studies suggest that placenta may have a specific mechanism of epigenetic regulation, different from that of embryonic tissues, differential H3K36me2 distributions helping to shape the divergent methylation patterns.

A TARGETED CRISPR/CAS9 SCREEN IDENTIFIES FOXA1 AS A KEY TRANSCRIPTION FACTOR INVOLVED IN MYC-DRIVEN AND DIET-DEPENDENT PROSTATE CANCER OUTCOMES

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Background: Accumulating evidence points to a role for high-fat diets and obesity in driving prostate cancer progression. Chromatin remodeling and epigenetic reprogramming are emerging mechanisms of prostate cancer progression, representing dynamic avenues through which a cell may respond to a high-fat diet or obese environment. However, the link between diet and chromatin-related processes in driving aggressive disease thus far has been uncharacterized.
Methods: Our experimental designs are articulated around a murine model of MYC-driven prostate cancer that recapitulates several molecular features of human prostate adenocarcinoma. To identify candidate chromatin- and epigenomic-related factors critical for prostate tumorigenesis, we performed CRISPR/Cas9 knock-out screens *in vitro* using custom CRISPR/Cas9 libraries targeting epigenetic-related genes. To validate the candidate genes, we generated stable knock-out cells using CRISPR/Cas9 and characterized their oncogenic phenotypes *in vitro* using a battery of cellular assays, *in vivo* under conditions of either a control diet (CTD; 10% kCal fat) or diet-induced obesity (DIO; 60% kCal fat), and through a series of next-generation sequencing-based experiments and analyses (*e.g.*, RNA-seq, ATAC-seq, ChIP-seq).
Results: Our *in vitro* CRISPR/Cas9 screen identified the pioneer transcription factor forkhead box A1 (FOXA1) as a top candidate in promoting MYC-driven prostate cancer cellular proliferation. Indeed, genetic knock-out of FOXA1 confirmed that FOXA1 contributes to prostate cancer cellular proliferation, as well as cell-cycle progression and the maintenance of mitochondrial respiration *in vitro.* Transcriptomic analysis further implicates FOXA1 in regulating metabolic processes such as glycolysis and fatty acid metabolism. Along this line, FOXA1 supports tumor initiation and growth under CTD *in vivo*. Critically, loss of FOXA1 abrogates DIO-dependent tumor growth.
Conclusions: Our studies suggest that FOXA1 supports diet-dependent MYC-driven tumorigenesis of the prostate. We expect the results of our molecular profiling experiments to further elucidate the ways in which diet dictates FOXA1 function, thereby influencing prostate cancer outcomes. By doing so, we would be

setting the stage for the use of targeted therapies that exploit aggressive prostate cancer's dependency on FOXA1 and other diet-dependent epigenetic factors.

CONTROL OF THE TRANSCRIPTIONAL LANDSCAPE BY TRA1 ACROSS YEAST SPECIES

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Tra1 is an essential component of the SAGA and NuA4 transcriptional co-activator complexes that regulates gene expression. In the budding yeast *S. cerevisiae*, loss of Tra1 function is associated with increased sensitivity to multiple stresses, including proteotoxic stress and treatment with antifungal drugs such as caspofungin and fluconazole. Tra1 mutants also display reduced chronological lifespan in *S. cerevisiae*, significantly altering the transcriptional landscape of the aging cell. We previously demonstrated that mutations in *TRA1* lead to increased sensitivity to caspofungin and reduced filamentation and pathogenicity in *C. albicans*. However, we recently found that, unlike in *S. cerevisiae*, loss of Tra1 function in *C. albicans* is associated with increased resistance to azoles. This indicate that Tra1 can differentially modulate the response to antifungal drugs across yeast species. Transcriptome analysis revealed that Tra1 mutations have a differential impact in *C. albicans* compared to *S. cerevisiae* Furthermore, while Tra1 mutant fails to induce filamentation upon treatment with serum, it displays unaltered morphogenetic transition in response to Hsp90 inhibition. This suggests that the requirements for Tra1 in *C. albicans* morphogenesis depends on the nature of environmental cues. Tra1 therefore emerges as a new complex regulator of the *C. albicans* dug resistance and pathogenicity and our data support transcriptional rewiring of the Tra1-regulated stress responses in *C. albicans*.

DISTINCT ENHANCER STATES DEFINE SUBGROUPS OF PRIMARY SYNOVIAL SARCOMA TUMOURS

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Synovial sarcoma (SS) is a soft-tissue malignancy that mainly affects young adults. SS is defined by a pathognomonic translocation between chromosome X and chromosome 18, resulting in the oncofusion protein, SS18-SSX. Unfortunately, conventional chemotherapeutics have failed to increase survival for patients. In normal cells, canonical SS18 is a member of two of the three major chromatin remodelling BAF complexes, cBAF and GBAF, critical for maintaining epigenetic control within cells. SS18-SSX can replace SS18 in cBAF and GBAF complexes and its integration within cBAF is associated with degradation of that complex, increasing the relative prevalence of GBAF. To further characterize the epigenetic state of SS we generated reference epigenomic maps for 52 pre-treatment primary SS tumours including ChIP-seq for 7 histone marks, whole genome methylation and RNA-seq. Unsupervised hierarchal clustering of active chromatin states (defined by H3K4me1/3 and H3K27ac) revealed two groups. Differential analysis based on enhancer location and motif enrichment suggests that these groups represent tumours harbouring distinct cBAF levels and thus we named these groups cBAF high and cBAF low, respectively. No significant difference in SS18-SSX RNA expression was identified across groups, although tissue microarrays from matched SS tumours demonstrated that the cBAF low group has a higher optical density following staining for the SS18-SSX oncoprotein compared to the cBAF high group. As a result, we hypothesize that in primary synovial sarcoma tumours, a high SS18-SSX oncoprotein level leads to a high GBAF:cBAF ratio, pathoepigenetically distinct from tumours with a low SS18-SSX oncoprotein level and a high cBAF:GBAF ratio**.** To test this hypothesis directly we altered the levels of SS18-SSX in a SS cell line model and are currently performing ChIP-seq analysis to determine if levels of SS18-SSX are associated with specific cBAF and GBAF associated enhancer states. To translate our findings, we have leveraged group specific enhancer RNAs (eRNAs) to generate a signature that can assign group membership from RNA-seq datasets alone. We are currently working to design a nanoString code set to validate our eRNA signature and enable patient stratification based on enhancer state. This stratification has direct relevance for emerging SS therapies targeting BAF complex dependencies in SS.

SEX-SPECIFIC DNA METHYLATION AND TRANSCRIPTOMIC ALTERATIONS IN LATE-GESTATION FOREBRAINS CAUSED BY AN EARLY PREIMPLANTATION ALCOHOL EXPOSURE

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Prenatal alcohol exposure is known to alter the epigenetic profiles of cells during brain development and is part of the molecular basis underpinning Fetal Alcohol Spectrum Disorder (FASD) etiology. However, the impact of alcohol exposure on the future epigenetic (DNA methylation and transcriptome) profiles of the forebrain remains mostly unknown, especially when this exposure occurs during the initial stages of development, before the embryo implants in the uterus. Our research hypothesis is that an alcohol exposure during preimplantation will initiate DNA methylation dysregulations during the embryonic epigenetic reprogramming wave and cause abnormal establishment of DNA methylation profile in the developing forebrain and alters gene expression profiles.
Our objectives are to identify if early embryonic ethanol-exposure leads to DNA methylation and transcriptomic errors in the

forebrain at late-gestation. To model early embryonic alcohol exposure, we subjected pregnant mouse females to ethanol at 2.5 days (E2.5), corresponding to embryos at the 8-cell stage. We collected E18.5 embryos and dissected the brains of ethanol-exposed and control (saline) embryos. We then established genome-wide quantitative DNA methylation profiles of forebrains samples (controls; 3 males and 3 females, ethanol-exposed; 5 males and 5 females) by Methyl-Seq and gene expression profiles by mRNA-seq followed by bioinformatics analyses. Based on our previous works on impact of embryonic alcohol exposure on the developing brain, we analyzed male and female samples independently. We uncovered 625 differentially methylated regions (DMRs; 100bp regions with >10% methylation differences) in male forebrains and 359 DMRs in female forebrains, with only 58 regions commonly affected in both sexes. While males have both increased and decreased methylation regions in ethanol-exposed forebrains, females present a majority of increased methylation regions in ethanol-exposed. Interestingly, we observed more differentially expressed gene ($p<0.05$) in female ethanol-exposed forebrains than in males (748 vs 372) and only 5 genes dysregulated in both sexes. Both gene ontology analysis of genic DMRs differentially expressed genes in male forebrains are related to synaptic transmission and neuron development, whereas gene ontology in female genic DMRs and differentially expressed genes reveals implication in cytoskeletal or embryo development and neuron development as well.
Our results show that an early acute alcohol exposure generates long-lasting sex-specific DNA methylation perturbations in the developing forebrains. Current analyses are being done to identify specific brain cell types that would be particularly vulnerable such an early exposure in order to better understand the mechanisms behind developmental and life-long consequences of early prenatal alcohol exposure.

CHROMATIN STATES ASSOCIATED WITH HISTONE ACETYLTRANSFERASE P300 AND NUCLEAR RECEPTOR SIGNALLING IN CELLULAR DIFFERENTIATION

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Chromatin states and epigenetic mechanisms command gene expression patterns in cellular differentiation, pivotal to tissue development and maintenance. For example, the sequential expression of myogenic regulatory factors including Myf5, MyoD and myogenin is essential for skeletal muscle formation and regeneration. Although the **transcriptional coactivator p300 is important for a myriad of cellular processes**[1], its intrinsic histone acetyltransferase (HAT) activity is critically required for MyoD and Myf5 gene expression and consequently for skeletal myogenesis. **Nonetheless, less is clear on how** p300 affects residue-specific histone acetylation in distinct chromatin state during cellular differentiation. Exploring embryonic and adult myogenic differentiation systems, we have shed lights on the impact of p300 HAT activity and histone acetylation on skeletal myoblast specification and differentiation in both normal and signal-enhanced milieu. We have reported that bexarotene, a clinically approved retinoid X receptor (RXR) agonist, is an efficient enhancer for the specification and differentiation of skeletal muscle lineage and promotes myoblast differentiation through the function of RXR as a transcription factor[2,3]. Mechanistically, RXR directly regulate the expression of muscle-specific proteins including Akt2 and muscle master regulator MyoD[3,4]. During the course of our comprehensive OMIC analyses, we have defined distinct chromatin state and residue-specific histone acetylation associated with p300 at the MyoD and myogenin regulatory loci in response to RXR signaling[4–7]. We have also found that RXR signaling sustains primary myoblast differentiation following pro-atrophic insult[3]. Taken together, our works have provided novel insights into the dynamics of chromatin state and epigenetic regulation in myogenesis. Many pathological conditions and diseases can cause muscle wasting, resulting in serious physical disability. Deciphering the molecular mechanisms underpinning skeletal myogenesis is a critical step in developing the best strategies to prevent and treat muscle physiopathology. Given the lack of pharmacotherapy to promote muscle regeneration for clinical application, RXR signaling presents a new avenue for dissecting the mechanisms of muscle regeneration in mammals.

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EXPOSURE OF GREENLANDIC INUIT AND SOUTH AFRICAN VHAVENDA MEN TO THE PERSISTENT DDT METABOLITE IS ASSOCIATED WITH AN ALTERED SPERM EPIGENOME AT REGIONS IMPLICATED IN PATERNAL EPIGENETIC TRANSMISSION AND DEVELOPMENTAL DISEASE

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Humans are exposed to a myriad of toxicants that have a multitude of negative health consequences including impacting generations to come. These generational effects were first described in animal models and epidemiological studies over 15 years ago and implicated germline epigenetic factors. However, the molecular mechanisms mediating this epigenetic inheritance remains almost entirely unexplored in humans. Exposure to the insecticide dichlorodiphenyltrichloroethane (DDT) is linked to birth defects, cancer, and neurodevelopmental delays (Turusov et al., EHP, 2002). Although DDT has been banned world-wide since the 1970s, it is still sanctioned for indoor residual spraying to control malarial disease vectors in Sub-Saharan Africa (WHO, 2006). The impacts of DDT on human health are not only restricted to regions of use since DDT and its metabolite *p,p'*-DDE are transported over long distances to Arctic regions by weather patterns and ocean currents (Staci et al., Science, 1995). In this study we studied two geographically diverse DDT and *p,p'*-DDE-exposed populations, Greenlandic Inuit and South African VhaVenda men, and aimed to determine whether sperm DNAme and chromatin enrichments were associated with levels of *p,p'*-DDE exposures and could be implicated in epigenetic inheritance. To quantitatively identify regions that were altered in DNAme and histone enrichment of exposed men, we used a sperm customized methyl-capture approach followed by sequencing (MCC-seq), and chromatin immunoprecipitation targeting histone H3K4me3 followed by sequencing (ChIP-seq). We then performed differential and functional analyses to investigate the relationship between DDT-associated alterations in the sperm epigenome, their predicted transmission to the embryo at fertilization and persistence throughout embryonic development. We identified genomic regions with altered DNAme (DNAme) and differential enrichment of H3K4me3 that were dose-like responsive to serum *p,p'*-DDE levels. Altered DNAme and H3K4me3 in sperm occurred at regulatory regions involved in fertility, disease, development and neurofunction. A subset of regions with altered sperm DNAme and H3K4me3 occurred at transposable elements and were predicted to persist in the pre-implantation embryo. These findings suggest that *p,p'*-DDE exposure in men may negatively impact embryo development and the health of future generations through epigenetic mechanisms.

CHROMATIN-GUIDED DNA METHYLATION: INSIGHTS FROM THE MOUSE GERMLINE & EARLY EMBRYO

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Crosstalk between histone methylation and DNA methylation (DNAme) plays an important role in gene regulation during normal development as well as in disease[1]. The H3K36 KMTases SETD2 and NSD1 for example, which deposit H3K36me3 and H3K36me2, are required for *de novo* DNAme in oocytes and prospermatogonia, respectively[2,3]. Pathogenic mutations in these KMTases, as well as PcG group proteins and *DNMT3A*, have been identified in many neurodevelopmental growth syndromes with similar phenotypes, including Sotos Syndrome, Luscan–Lumish syndrome, Weaver syndrome and Tatton-Brown–Rahman syndrome, each of which show aberrant DNAme profiles in peripheral blood. The intimate interplay between H3K36me2/3 and DNAme is also apparent in cancers in which H3K36 KMTases are mutated or overexpressed. Recurrent mutations in NSD1 for example, which are observed in lung as well as head and neck squamous cell carcinoma, result in loss of DNAme, while pathogenic SETD2 mutations in clear cell renal cell carcinoma are associated with loss of DNAme. Notably, pathogenic mutations in the catalytic domain of DNMT3A, which result in loss of DNAme, are also observed in various cancers and may be causal of malignant transformation in AML. Thus, as for neurodevelopmental growth disorders, disruption of H3K36me2 or H3K36me3 deposition is likely to have an important role in tumorigenesis at least in part by perturbing DNAme homeostasis. However, negative crosstalk between H3K36me2/3 and the PRC1 and/or PRC2 PcG complexes or their catalytic activities (H2AK119 ubiquitination and H3K27 methylation, respectively), are also likely to play a role in the etiology of these diseases. I will discuss our recent findings, using the mouse germline and early embryo as model systems, on the complex crosstalk between histone methylation and DNAme broadly and over CpG island promoters[4], and the impact of these interactions on the transcriptome.

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A PROSPECTIVE STUDY OF TIME-DEPENDENT CHILDHOOD ADVERSITY AND DNA METHYLATION ACROSS CHILDHOOD AND ADOLESCENCE

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Background: Childhood adversity (e.g., abuse, poverty, etc.) is one of the most potent determinants of adverse health outcomes. Recent evidence also suggests that these stressful events may be particularly detrimental during *sensitive periods*, when physiological systems may have heightened susceptibility to external influences. However, the mechanisms linking childhood adversity to long-term health outcomes are not yet fully understood. Although emerging evidence suggests that childhood DNA methylation (DNAm) may capture some time-dependent effects of early life adversity, it remains unclear

whether these epigenetic alterations persist into adolescence and how the timing of adversity might influence their developmental trajectories. In this study, we examined the relationship between the timing of childhood adversity and genome-wide DNAm measured at three waves from birth to adolescence using data from the Avon Longitudinal Study of Parents and Children (n=970).

Methods: We assessed the relationship between the timing of exposure to seven types of adversity (prospectively measured 5-8 times between ages 0-11) and DNAm at ages 7 and 15. First, we used the Structured Life Course Modeling Approach (SLCMA) to identify associations between exposures to adversity in very early (age 0-2), early (3-5), middle (6-8), or late (9-12) childhood and DNAm patterns at age 15. Next, we characterized the persistence into adolescence of associations previously identified in age 7 DNAm, as well as the impact of adversity on DNAm trajectories from ages 0-15.

Results: Adversity exposure was associated with differences in age 15 DNAm at 22 loci (FDR<0.05), which were distinct from age 7 associations. Most loci (16 of 22) were associated with adversities occurring between ages 3-5 (i.e., physical/sexual abuse, one-adult households). DNAm differences present at age 7 did not persist into adolescence. These findings were robust in internal validation analyses using nonparametric bootstrapping. Six distinct types of DNAm trajectories were characterized for these top loci, which highlighted both immediate and latent effects of adversity.

Conclusions: Childhood adversity has immediate and latent effects on DNAm profiles across development, particularly when exposures occur between ages 3-5. These findings highlight potential sensitive periods and biological mechanisms that can be leveraged to predict and prevent the physical and mental outcomes linked to adversity.

CHARACTERIZING THE EPIGENOME OF MELANOMA SUBTYPES TO REVEAL INSIGHTS INTO IMMUNE THERAPY RESPONSE

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Melanocytes are found in the basal layer of the epidermis, but also reside throughout the human body, and are the cell of origin of melanomas. As a result, melanomas arise most commonly in sun exposed skin, but also in uvea of the eye and mucosal membranes. A subtype called acral melanomas also develop in non-sun exposed skin of the palms of the hands and soles of the feet as well as underneath nail beds. Interestingly, melanomas from these various anatomical sites acquire different sets of driver mutations. Part of this melanoma-subtype specific accumulation of mutations may be due to differences in sun exposure of melanocytes, but may also be attributable to the cell of origin of melanocytes and their epigenetic states while residing in diverse microenvironments (*e.g.* skin, uvea, and mucosal membranes). Furthermore, cutaneous melanomas have high response rates to immune checkpoint inhibitors (ICIs), but responses are less common in mucosal and uveal melanomas. Although significant effort has been made in determining mutations that correlate with therapy resistance, few studies have examined the role of the epigenome in ICI response. To address whether different melanoma subtypes exhibit different epigenetic states, we performed a multi-omic analysis with epigenome (DNA methylome) and transcriptome profiling of 483 cutaneous, 113 uveal, 63 mucosal, and 49 acral melanomas. Specifically, we examined subtype-specific differences in DNA methylation and transposable elements (TEs) expression, a mechanism of viral mimicry linked to the immune response in other cancers. We observed uveal melanoma exhibited a completely different DNA methylation pattern compared to the other melanoma subtypes, whereby cutaneous, acral and mucosal melanomas were indistinguishable from each other. Furthermore, loss of epigenetic regulation leading to up-regulation of TEs (primarily LTR and Alu classes) was most commonly found in cutaneous melanomas, and least in uveal melanomas which have the lowest ICI response rates amongst melanoma subtypes. In summary, this work revealed a unique epigenetic state found in uveal melanomas which also have the lowest levels of TE expression compared to the other melanoma subtypes. These results provide insights into the different melanoma-specific differences in the epigenome which may provide insight into different ICI responses observed in uveal melanoma.

H3K36 REGULATOR NSD2 DYNAMICS AND FUNCTION IN THE NUCLEOLUS

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Nuclear receptor-binding SET domain protein 2 (NSD2) is a histone lysine methyltransferase that deposits H3K36me2 mark to regulate chromatin, transcription, and the DNA damage response. The t(4; 14) translocation, found in 15-20% of multiple myeloma patients, leads to high expression of NSD2 and is associated with unfavorable prognosis. Depending on the location of the t(4;14) breakpoints in myeloma cells, NSD2 forms with N-terminal truncations lacking the PWWP1 reader domain are often generated. Interestingly, truncated NSD2 forms display a prominent nucleolar localization. Similarly, inhibition of PWWP1 domain with the chemical probe UNC6934, promotes the accumulation of NSD2 in the nucleolus. Whether truncated NSD2 isoforms play differential roles associated with nucleolus function remains to be determined.

To better understand the dynamics of NSD2 in the nucleolus, we performed fluorescence recovery after photobleaching (FRAP). FRAP data indicates that truncated NSD2 shuttles between the nucleolus and the nucleus, displaying a slower recovery time after photobleaching when compared to nucleolar signal alone or fibrillarin. Additionally, mutations at the c-terminal nucleolar sequences were not sufficient to abrogate NSD2 nucleolar localization. To gain insight into the regulation and biological

relevance of NSD2 nucleolar localization, we compared full length and N-terminal truncated NSD2 interactomes using the proximity-based biotin labeling, BioID. Nucleolar NSD2 lost the interaction with bromo domain and extra-terminal motif proteins BRD2 and BRD4. However, bromodomain adjacent to zinc finger domain protein BAZ family members preferentially interacted with the nucleolar NSD2. Numerous other chromatin regulators were also found to interact with the nucleolar form of NSD2, suggesting an organelle-specific interactome rewiring. Importantly NSD2 regulated H3K36 methylation of the ribosomal DNA and regulatory noncoding RNA loci. Additionally, knockout or targeted degradation of NSD2 significantly reduced ribosomal RNA synthesis. Together, our work indicates that NSD2 plays a key role in the regulation of the ribosomal DNA synthesis and integrity.

CELL-FREE CHROMATIN PROFILES FROM PRECLINICAL MODELS REFLECT MOLECULAR PHENOTYPES

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Cell-free chromatin is a rich source of biomarkers across a range of conditions, including cancer. Tumor-derived nucleosomes enter the circulation where they can be noninvasively accessed for epigenetic profiling. Genome-wide patterns of nucleosome positioning and histone modifications reveal chromatin organization and gene expression reflective of distinct cellular phenotypes. In most cancer patients, circulating tumor-derived nucleosomes are far outnumbered by those from other cell types, presenting challenges for using blood plasma for efficient and reproducible biomarker discovery. Here, we describe a robust and generalizable framework for generating cell-free nucleosomes from cell line conditioned media. Sequencing-based analysis of cell-free nucleosome positioning demonstrates consistent depletion at transcriptional start sites of active genes. Patterns of nucleosomes associated with active and repressive histone modifications reveal differential epigenetic states from the cell line of origin. Our results demonstrate the potential of preclinical simulation of cell-free chromatin for the discovery of epigenetic signatures for use in liquid biopsy.

EPIGENETIC AGE DYSREGULATION IN INDIVIDUALS WITH BIPOLAR DISORDER AND SCHIZOPHRENIA

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Bipolar disorder (BD) and schizophrenia (SCZ) are psychiatric disorders often associated with psychosis and present a significant societal burden and reduced quality of life for affected individuals and their families. Despite having a significant heritable component, the presentation and course of these disorders vary tremendously among affected individuals and across the lifespan. The delayed age of onset suggests an association with the temporal dimension of aging. Epigenetic clocks can be used to calculate biological age, which can serve as a biomarker of age-related physiological decline.
In this study, we used microarray data derived from both the Illumina HumanMethylation450 (450K) and MethylationEPIC (EPIC) platforms to compute the epigenetic age of individuals with SCZ (n = 40), BD (n = 40), age- and sex-matched healthy controls (n = 38), using five epigenetic clocks. Various statistical metrics were used to identify discrepancies between epigenetic and chronological age across the three groups.
We observed significant differences in chronological compared to epigenetic ages estimated by the Horvath clock in the BD (p = 0.00046) and SCZ (p = 0.0066), but not control groups (p = 0.16). Strong and significant correlations between chronological age and epigenetic age were observed between two variables across all tested clocks and groups (Spearman's rho > 0.5 and < -0.5, $p < 8 \times 10^{-6}$). Comparing the mean residuals derived from the Spearman correlations, significant, groupwise differences for Horvath ($p = 9.7 \times 10^{-6}$), Horvath Skin & Blood ($p = 8 \times 10^{-11}$), Hannum ($p = 2.6 \times 10^{-8}$), and PhenoAge ($p = 2.5 \times 10^{-9}$) clocks were observed.
Despite the study's relatively small sample size, these findings suggest that both individuals with BD and SCZ may have epigenetic markers associated with a dysregulated aging phenotype, which could be suggestive of negative outcomes associated with the disease. In future studies, we hope to further elucidate these findings by demonstrating the precise link between epigenetic age, cell type composition, symptomatology, and disease progression.

JAZF1 LINKS THE EPIGENETIC FUNCTION OF THE NUA4/TIP60 ACETYLTRANSFERASE COMPLEX TO THE MTOR METABOLIC SIGNALING PATHWAY

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The human multisubunit histone acetyltransferase complex NuA4/TIP60 acts as an epigenetic factor and gene co-activator via acetylation of histones H4/H2A and H2A.Z variant deposition, thereby facilitating transcription. TIP60 utilizes acetyl-CoA, a key nutrient-sensitive metabolite, as a source of acetyl groups for modification of its histone targets, bridging cellular metabolism and transcriptional regulation. Recently, TIP60 has been reported to associate with JAZF1, a poorly characterized transcriptional regulator that has been extensively linked to metabolic homeostasis in mammalian models. The Saccharomyces cerevisiae ortholog of JAZF1 also plays a central role in budding yeast metabolism and is known to be modulated by the TORC1 growth-regulating kinase. Here, we demonstrate that JAZF1 is a stable subunit of the TIP60 complex by mass spectrometry following tandem affinity purification. Through RNA-seq against a

knock-out background, we find that JAZF1 rewires the metabolic transcriptome, resulting in upregulation of genes involved in lipid metabolism and mTOR signaling. Strikingly, RICTOR, the gene coding for an mTORC2-specific subunit, is at the top of upregulated genes, suggesting crosstalk with the mTOR pathway at the transcriptional level. The majority of upregulated genes, including RICTOR, are direct targets of JAZF1, as evidenced by correlation with JAZF1 binding sites identified by CUT&RUN. Integrated RNA-seq and CUT&RUN analyses in the absence or presence of JAZF1 reveal that JAZF1 enhances TIP60's activity but not binding at target metabolic genes. Current work is aimed at elucidating the molecular mechanisms, specifically potential post-translational modifications, mediating the functional crosstalk between JAZF1-TIP60 and mTOR. Altogether, our study adds more nuance to TIP60's implication in metabolic-epigenetic crosstalk, describing its novel subunit JAZF1 as an effector and modulator of a major metabolic signaling pathway.

GLOBAL REPRESSION AND VARIABLE COMPENSATION FOLLOWING INHIBITION OF MAMMALIAN SWI/SNF

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The mammalian SWI/SNF chromatin remodeling complexes move and reorganize nucleosomes at gene promoters and enhancers to modulate access to DNA. Although SWI/SNF subunits are commonly mutated in disease, therapeutic options are limited by a lack of mechanistic insight into the direct targets and effects of SWI/SNF. While both activating and repressive roles have been described for the complex, recent work with pharmacological inhibitors suggests that the primary role of SWI/SNF is to promote chromatin accessibility and transcription factor binding, particularly at enhancers. However, cells subjected to long term SWI/SNF inhibition or protein depletion show only a modest subset of genes to be affected, raising questions about the variable impact of SWI/SNF remodeling on gene activity.
Here, we use a fast-acting catalytic inhibitor of the SWI/SNF ATPases to address these questions in mouse embryonic stem cells. We find that inhibition of SWI/SNF rapidly causes a global loss of chromatin accessibility at both promoters and enhancers, concomitant with widespread suppression of transcription initiation. However, most promoters can regain accessibility and reactivate within hours, in the continued absence of SWI/SNF remodeling. We identify EP400/TIP60 as the key factor mediating this compensation, and connect promoter recovery to the presence of H3K4me3, a histone mark bound by EP400/TIP60. Accordingly, a majority of enhancers and a set of cell type-specific genes with low expression are depleted for H3K4me3 and fail to recover accessibility or transcription activity following SWI/SNF inhibition. Consistent with an increased importance of EP400 when SWI/SNF function is impaired, analysis of patient cancer data reveals that mutations in EP400 are mutually exclusive with mutations in SWI/SNF subunits.
Together, our findings demonstrate a universal role for SWI/SNF in generating accessible chromatin that is masked by compensation during long-term SWI/SNF disruption. We note that widespread reestablishment of promoter activity in the absence of appropriate enhancer recovery implies disruption of promoter-enhancer communication upon SWI/SNF perturbation, providing insights into disease. Further, the molecular characteristics defining SWI/SNF-dependence at promoters in mouse embryonic stem cells can predict which genes will be sensitive to SWI/SNF inhibition in human cancer cells.

THE IMPACT OF SARS-COV-2 ON THE MATERNAL-FETAL INTERFACE THROUGH SINGLE CELL TRANSCRIPTOMICS AND EPIGENOMICS

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Pregnancy outcomes are dependent on normal placental function. The placenta consists of diverse cell types that originate from both the fetus and the mother, which work together to maintain and develop the organ. Recent studies have reported adverse pregnancy outcomes associated with SARS-CoV-2 infection, despite the lack of SARS-CoV-2 detected in the placenta. However, the molecular mechanisms responsible for these and other traits remain elusive. To delineate the impacts of SARS-CoV-2 infection, we profiled the transcriptomes and epigenomes of maternal-fetal interface tissue from COVID-19 patients at single nuclei and bulk resolutions. Our results reveal an imbalanced angiogenesis and inflammatory response in fibroblast and endothelial cell types. Additionally, we also find a hyperactive innate immune response in immune cell types at the maternal-fetal interface. The transcriptomic dysregulation is accompanied by chromatin accessibility changes at annotated *cis*-regulatory elements. Furthermore, we also discovered new predicted cell to cell interactions in inflammatory and innate immune activation pathways in COVID-19 patients. Intriguingly, our results show a dysregulation of retrotransposons, which are mobile genomic sequences that can possess *cis*-regulatory activities and influence transcription. In particular, endogenous retroviruses are enriched among these elements. We found reduced activity of LTR8B elements which is functionally linked to decreased expression of Pregnancy Specific Glycoprotein (PSG) genes and other pregnancy related genes in syncytiotrophoblast cells of COVID-19 patients. Overall, we demonstrate the involvement of non-coding sequences in dysregulation of pregnancy-related genes, angiogenesis pathways and innate immune response at the maternal-fetal interface of COVID-19 patients.

PERSISTENT ACETYLATION OF NEW HISTONES INHIBITS THE ACTIVATION OF ORIGINS OF DNA REPLICATION

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Histone acetylation is a known regulator of DNA replication dynamics. In the yeast *Saccharomyces cerevisiae*, newly synthesized histone H3 deposited behind DNA replication forks are acetylated on lysine 56 (H3 K56ac) by the Rtt109 histone acetyltransferase. Two enzymes of the sirtuin family of deacetylases, Hst3 and Hst4, act redundantly to deacetylate this residue throughout chromosomes after S phase. Cells lacking Hst3 and Hst4 present constitutively acetylated H3 K56, which causes sensitivity to replication-blocking genotoxins and slow growth via mechanisms that remain poorly understood. Here, we present the results of a genome-wide screen aimed at identifying haploinsufficient genes that promote cell fitness in response to nicotinamide (NAM) induced inhibition of sirtuins. We find that heterozygosity of genes involved in the regulation of DNA replication origins sensitize cells to NAM. Consistently, haploid cells harboring the hypomorphic temperature sensitive allele *cdc7-4* present striking NAM-induced S phase progression defects at their semi-permissive temperature. We further show i) that Rap1-interacting factor 1 (Rif1), an inhibitor of Cdc7-dependent activation of replication origins, causes DNA damage and replication defects in cells exposed to NAM and in *hst3Δ hst4Δ* mutants, and ii) that *cdc7-4 hst3Δ hst4Δ* cells present strong synthetic temperature sensitivity associated with defective activation of replication origin and S phase progression delay. Our data further demonstrate that such replication defects are not due to intra-S phase checkpoint activation leading to inhibition of origin activity. Finally, we show that Rtt109-dependent acetylation of histone H3 lysine 56 and its associated Rtt101-Mms1 ubiquitin ligase complex cause the DNA replication defects observed in *cdc7-4 hst3Δ hst4Δ* cells. Overall, the above results argue that the abnormal regulation of nascent chromatin structure negatively influences DNA replication initiation in cells presenting reduced DDK activity such as those experiencing replicative stress.

CRISPR DELETION PROVIDES MECHANISTIC INSIGHT INTO GENE EXPRESSION REGULATION BY DISTAL ENHANCERS

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Non-coding transcriptional enhancers are critical for development, phenotype divergence during evolution and often mutated in disease contexts; however, even in well-studied cell types, the sequence code conferring enhancer activity and the mechanisms through which they regulate specific genes remains unknown. Enhancers are key drivers of pluripotency maintenance and the reprogramming process; therefore, determining the repertoire of sequences and transcription factors that confer activity to these regions will provide a better understanding of the pluripotent state and reveal transcriptional control mechanisms that define cell identity. Using CRISPR deletions we identify and study the mechanisms through which cis-regulatory sequences regulate transcription of their target genes. This approach has revealed a surprising disconnect between common approaches to identify enhancers using chromatin features and sequences with regulatory function.

THE ROLE OF M6A RNA METHYLATION IN SEX-INFLUENCED MOLECULAR CHANGES IN DEPRESSION

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Introduction: Females are twice as likely to be diagnosed with Major Depressive Disorder (MDD) compared to males. This is a striking example of sex differences in MDD, and mounting evidence suggests that it may be driven by sex-specific molecular mechanisms. Mounting evidence suggests that epigenetic alterations in response to environmental stimuli contribute to the pathology of MDD. However, little is known about the role of equivalent chemical modifications in RNA molecules. In recent years, RNA modifications have emerged as a dynamic and crucial mechanism in the post-transcriptional regulation of gene expression. Among 150 known RNA modifications, N6-methyladenosine (m6A) is the most abundant RNA modification in protein-coding mRNA. Emerging evidence suggests that m6A plays an important role in the brain, including neurodifferentiation, neurogenesis, and memory and learning. Moreover, recent studies have linked m6A to molecular and behavioral responses to stress, making it an important candidate regulator of stress-related psychiatric disorders, including MDD. Given the known sex difference in stress response, this study aims to identify m6A profiles associated with sex-influenced molecular changes in MDD.
Methods: To investigate m6A landscape at transcripts levels and the impact of m6A on gene expression, m6A-seq and RNA-seq were conducted on the ventromedial prefrontal cortex obtained from male and female MDD and healthy control subjects.
Results: Using our optimized m6A-seq protocol, we identified ~25,000 m6A peaks in the human brain, and these peaks were enriched in the known m6A consensus motif "GGAC" and 3'UTR and coding region. Also, these m6A tagged genes were related to neuronal and synaptic regulation as suggested by previous studies. Interestingly, our differential m6A analysis shows a distinct m6A profile in MDD and control, with a little overlap between males and females. However, these differentially methylated genes were enriched for genes related to synaptic function in both male and female with MDD.
Conclusion: Our results highlight a significant role of m6A in MDD, possibly by adjusting the consequences of synaptic-related genes. Further analysis will help us understand the role of m6A in

stress-related psychiatric disorders and will serve as an example of sex-specific analysis in MDD.

REPRESSION OF GERMLINE GENES BY PRC1.6 AND SETDB1 IN THE EARLY EMBRYO PRECEDES DNA METHYLATION-MEDIATED SILENCING

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Silencing of a subset of germline genes is dependent upon DNA methylation (DNAme) post-implantation. However, these genes are generally hypomethylated in the ICM, implicating alternative silencing pathways before implantation. Indeed, in embryonic stem cells (ESCs), an overlapping set of genes, including germline "genome-defense" (GGD) genes, are upregulated following deletion of the H3K9 methyltransferase SETDB1 or subunits of the non-canonical PRC1 complex PRC1.6. Here, we show that in pre-implantation embryos and naïve ESCs (nESCs), hypomethylated promoters of germline genes bound by the PRC1.6 DNA-binding subunits MGA/MAX/E2F6 are enriched for RING1B-dependent H2AK119ub1 and H3K9me3. Accordingly, silencing of these genes in nESCs shows a greater dependence on PRC1.6 than DNAme. In contrast, GGD genes are hypermethylated in epiblast-like cells and their silencing is dependent upon SETDB1, PRC1.6/RING1B and DNAme, with H3K9me3 and DNAme establishment dependent upon MGA binding. Thus, GGD genes are initially repressed by PRC1.6, with DNAme subsequently engaged in post-implantation embryos.

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 the 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.
IHEC has produced a total of 648 WGBS reference datasets, of which 588 have been released to date. However, over half of these datasets fall below the 30x coverage cut-off used by the IHEC standards. As such, we explored if we could impute missing CpG sites within these low-coverage datasets to improve these valuable datasets. For this, we benchmarked multiple imputation tools, including DeepCpG (Angermueller et al. 2017), BoostMe (Zou et al. 2018) and GIMMEcpg (Moghul et al. *in prep*). These tools utilise deep learning or machine learning techniques to learn from neighbouring CpG sites within the same dataset to impute missing CpG sites. All low-coverage WGBS were successfully imputed to reference-grade IHEC WGBS. The remaining datasets will be imputed once they become available.
Moreover, the vast majority of IHEC datasets do not include WGBS data, drastically decreasing the number of complete reference epigenomes within the IHEC dataset. Tools like ChromImpute can impute WGBS data using other epigenetic marks (e.g., histone data). Therefore, we explored and benchmarked ChromImpute to determine its accuracy for imputing WGBS. The initial results indicate that it is possible to impute methylation data at very high accuracy (MAE: 7.6-10%; R: 0.85-0.95). Furthermore, we are currently exploring which histone mark is driving the methylation signal.
Based on these results, we anticipate that imputing methylomes will help bring the number of complete human reference epigenomes from 346 to over 2500. In conclusion, we show that imputation is a powerful tool for boosting the quality of existing low-coverage datasets as well as for datasets without any WGBS data altogether.

SURVIVIN AND CASPASES COORDINATE PROTEOLYTIC CLEAVAGE OF THE SAGA CHROMATIN MODIFYING COMPLEX

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The Spt Ada Gcn5 Acetyltransferase (SAGA) chromatin modifying complex is a critical regulator of gene expression. Mutation or stoichiometric imbalance of SAGA subunits leads to a spectrum of diseases in model organisms and in humans, including neurodegeneration. However, delineation of gene sets misregulated upon SAGA disruption is yet to reveal pathways suitable for therapeutic targeting.
SAGA contributes to gene activation through coordinated function of intrinsic histone-acetyltransferase and deubiquitinase activities which are housed within distinct, functionally independent, modules. Although much is understood about how acetylation effects SAGA function, mechanisms regulating SAGA deubiquitinase module are more mysterious. Recently, we discovered the SAGA deubiquitinase module can be released from SAGA to interact with other multi-protein complexes, such as WAVE Regulatory and Arp2/3 complexes which function together to control actin branching (Cloud et al., 2019).
The pathways that regulate deubiquitinase module entry and exit from SAGA remain mysterious. In human cells, Caspase 7 irreversibly post-translationally modifies SAGA through proteolytic cleavage. Our analysis predicts Caspase cleavage will release the deubiquitinase module from SAGA. Preventing Caspase -mediated cleavage of SAGA reduces cytotoxicity associated with a mutation of SAGA that causes

neurodegeneration. Therefore, it is of therapeutic interest to identify pathways working to prevent proteolytic cleavage of SAGA.
We performed a biochemical screen to identify novel interactors of SAGA. This revealed the survivin protein. Survivin possesses a Baculoviral IAP Repeat (BIR) domain characteristic of Inhibitor of Apoptosis Proteins (IAP). IAPs function as decoys by competitively binding to caspases via their BIR domains, locally preventing caspase-mediated cleavage. SAGA bears two caspase cleavage sites on SAGA subunit Atxn7. Preliminary data show loss of survivin results in proteolytic cleavage of Drosophila Atxn7 similar to that seen when mammalian Atxn7 is proteolytically cleaved by Caspase-7.

DEFINING GATA-4/5/6 REGULATORY LOGIC DURING EARLY HEART DEVELOPMENT

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Embryogenesis is a tightly controlled process driven by exquisite activation of conserved gene regulatory networks. In heart development, GATA4, GATA5, and GATA6 transcription factors sit high up in the regulatory hierarchy. Using morpholino-mediated Gata5/6 knockdown (KD) in zebrafish we previously found GATA-sensitive chromatin accessibility changes in *gata5* expressing cells at 8hpf, a developmental timepoint prior to the onset of conventional cardiac progenitors. In this study we set out to understand the temporal dynamics of this GATA-dependent chromatin accessibility by profiling accessibility in *gata5* expressing cells throughout early development from early gastrulation to early segmentation stages (6hpf-13hpf). We find that many GATA-sensitive differentially accessible regions (gsDARs) identified at 8hpf are inaccessible at 6hpf suggesting a narrow window of GATA activity between 6-8hpf to establish cardiac regulatory networks. While many of these gsDARs display cardiac activity at heart forming stages, the genes these elements regulate have yet to be established. To identify target genes, we used a chromosome conformation capture approach. Of 17 assayed gsDARs we were able to detect contacts with genes in 16 cases. Similarly, we identified gsDARs with cardiac promoter viewpoints, detecting contacts from the promoters of *tbx1, tbx20*, *hand2*, *zfpm1,* and *zfpm2a*. To see if changes in accessibility at these sites also reflected changes in enhancer-promoter wiring, we examined contact frequencies in the absence of Gata5/6. Of the 22 viewpoints, 3 had minor but significant changes in contact frequency when Gata5/6 are lost. In 10 instances we observed widespread changes in contact frequency across the locus with an increase in contacts on one side of the viewpoint and a corresponding decrease on the other side - suggesting a potential creation of novel weak chromatin domain boundaries. Lastly to understand the relevance of these gsDARs on gene activity in cardiac mesoderm development, we are currently performing Single Cell Multiome ATAC + Gene Expression at 8hpf in WT and Gata5/6 KD *gata5* expressing cells. By constructing gene regulatory networks, we have started to identify promising candidate elements, including a novel cardiac-specific regulatory element for *gata5*. These results highlight the essential contribution of Gata factors to gene regulatory networks that govern the earliest stages of heart development.

THE CHROMATIN REMODELING FACTOR CREST DIRECTS ESTABLISHMENT OF SYNAPTIC ENHANCERS IN MATURING MOTOR NEURONS

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ATP-dependent chromatin-remodeler BAF complexes have specific subunits required for gene expression in post-mitotic neurons. CREST is a post-mitotic neuron-specific BAF subunit that regulates genes essential for dendritic outgrowth in various neuronal cell types. Mutations in CREST have been identified in individuals with ALS motor neuron disease. How CREST regulates expression of neuronal genes in maturing spinal motor neurons remains largely unknown. Using *CREST* knockout cell lines combined with an efficient mouse stem cell differentiation method, we discovered that genes related to synaptic signaling and neural development are significantly downregulated in *CREST* knockout cells. Differentially expressed genes in maturing *CREST* knockout cells showed globally similar gene expression levels as nascent wildtype motor neurons. These results imply that CREST is involved in the expression of genes important for the transition from nascent to maturing motor neurons. We also revealed that enhancers linked to synaptic neuronal genes are depleted of H3K27ac-enrichment in *CREST* knockout cells compared to wildtype cells. These results indicate that CREST contributes to the establishment of enhancers linked to synaptic genes. CREST may play a role in neuron-specific gene expression programs and the maintenance of spinal motor neuron identity.

MONOALLELIC ENHANCER DELETIONS DISRUPT H3K27AC, OPEN CHROMATIN, AND NON-CODING RNA AT DEFINED TARGET LOCI

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Enhancers are non-coding genomic regions that positively regulate the expression of one or more target genes to instruct complex processes like differentiation, responses to environmental stimuli; or drive abnormal transcription in certain disease contexts. To date, cataloguing and annotating enhancer elements by target gene and function has proven difficult because they lack a reproducible genetic signature. Enhancers can act bidirectionally and at far distances from their gene target and participate in multi-way enhancer-promoter interactions. Currently enhancer sequences are most accurately predicted by integrating functional genomic and epigenetic features such as transcription factor binding, chromatin accessibility, enhancer RNA production, and histone modifications such as Histone H3 lysine 27 acetyl (H3K27ac). High false positive rates remain a

shortcoming of contemporary algorithms for enhancer prediction. My studies aim to address a major gap in our understanding of the relationship between enhancer function and the chromatin features frequently associated with, but not restricted to, enhancer regions. Using a hybrid F1 (*M. musculus*/129 × *M. castaneus*) mouse embryonic stem cell (ESC) line and *Sall1* as a model locus regulated by an enhancer cluster, I have performed allele-specific enhancer deletions to assess the contribution of active enhancers to adjacent yet non-regulatory genomic regions displaying enhancer-associated features that could be categorized as false positive enhancer predictions. Bulk allele-sorted ATAC-seq, allele-specific ChIP-qPCR and short non-coding RNA expression profiling revealed that active enhancers can induce H3K27ac enrichment, chromatin accessibility, and enhancer-like RNA production at defined target loci over 100 kb away. These loci responsive to *Sall1* enhancer deletion exhibit occupancy by a small number of pluripotency transcription factors, which show a subtle reduction of binding in deletants. Collectively, my data indicate that active enhancers change the chromatin landscape, particularly enhancer-associated features, by influencing local transcription factor recruitment. This work will help build our understanding of the interplay between functional enhancers and their chromatin context to help improve the accuracy of transcriptional enhancer predictions.

NON-REDUNDANT REGULATORY ROLE OF H3K27ME3 DEMETHYLASES JMJD3 AND UTX UNVEIL PRO-REGENERATIVE FUNCTION OF HYALURONAN IN MUSCLE REGENERATION.

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Chromatin landscape decorated by various modifications on histones regulates cell- and tissue-specific gene expression. Histone trimethylation, specifically on histone H3 lysine 27 (H3K27me3) impedes spurious transcription and regulates spatio-temporal expression of lineage-determination genes. While the enzymatic enhancer of zeste homolog 2 (EZH2) of Polycomb Repressive Complex 2 (PRC2) engages in trimethylation of H3K27, the jmjc-domain containing KDM6 family proteins UTX (KDM6A) and JMJD3 (KDM6B) are required for its demethylation and expression of lineage-specific genes. Nevertheless, it is unclear how distinct demethylases for a particular histone modification spatially coordinate and dictate cell fate. To decipher the divergent functional regulatory roles, we characterized satellite stem cell-mediated muscle regeneration as muscle stem cells (MuSCs) express both UTX and JMJD3 in quiescence. MuSC-specific inducible knockout mice of UTX (UTX^{scKO}) and JMJD3 ($JMJD3^{scKO}$) were generated. Upon cardiotoxin-induced acute muscle injury and single-cell RNA-sequencing analysis, we found that unlike UTX, which is required for terminal differentiation of MuSCs, JMJD3 is required for early activation and cell-cycle re-entry of MuSCs. Using damaged muscle extract on single myofibers, we demonstrated that JMJD3 is typically required for MuSC activation only in the context of an inflammatory niche. Transcriptome analysis of MuSCs lacking UTX and JMJD3 at 30 hrs postinjury revealed that there exists an unresolved cytokine storm in $JMJD3^{scKO}$ MuSCs. Genome-wide profiling of the demethylases JMJD3 and UTX along with H3K27me3 in $JMJD3^{scKO}$ and UTX^{scKO} MuSCs by Cleavage Under Target and Tagmentation (CUT&Tag), we have identified *Has2* as a key contributor of JMJD3-mediated MuSC activation. $JMJD3^{scKO}$ MuSCs lack hyaluronic acid (HA) on their surface and inhibition of hyaluronic acid synthesis using 4-Methyl Umbelliferone (4-MU), impaired activation/cell-cycle re-entry of wild-type MuSCs. Furthermore, our studies revealed that upon injury, pro-inflammatory cytokine IFN impedes $JMJD3^{scKO}$ MuSC activation and exogenous supplementation of hyaluronic acid into culture media containing single myofibers can rescue the activation defect. Overall, we demonstrated a novel JMJD3-*Has2*/HA-IFN axis that integrates extracellular niche signals and primes MuSCs to exit quiescence and initiate muscle regeneration. Thus, these studies for the first time provide evidence of non-redundant regulatory roles of H3K27me3 histone demethylases UTX and JMJD3 in the same cell.

HIGHER-ORDER CHROMATIN ORGANIZATION IN PROGERIA VASCULAR SMOOTH MUSCLE CELLS

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare human genetic disorder caused by mutations in the LMNA gene, which results in a severe premature aging phenotype. HGPS is caused by accumulation of Progerin, an abnormal form of Lamin A that leads to fatal atherosclerosis and acute loss of vascular smooth muscle cells (VSMCs). Cellular features of HGPS include disruption of nuclear scaffolding causing nuclear blebbing, loss of peripheral heterochromatin, defective epigenetic inheritance, altered gene expression, and accelerated aging. Additionally, the widespread distribution of Lamin A suggests that it may play a role in chromatin compartmentalization in the nucleus. Here, we used HGPS patient-derived VSMCs to better understand the impact of chromatin structure on regulation of HGPS transcriptional profile. By combining high-throughput chromosome conformation capture (Hi-C) and transcriptome profile, we identified changes in topologically associated domains (TADs) that lead to alterations in HGPS transcriptome. We found that in the early stages of HGPS progression there is re-organization of chromatin compartments causing dysregulation of aging hallmarks. Furthermore, in the late stages of HGPS we observed TAD alterations and possible gene-enhancer miscommunication in genes involved in extracellular matrix and cardiovascular diseases. Our results therefore reveal that the non-

randomly compacted three-dimensional chromatin structure has instructive roles in regulating gene expression in HGPS.

TFIID SUPPORTS RNA POLYMERASE II TRANSCRIPTION INDEPENDENTLY OF TBP

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Transcription by RNA Polymerase II (Pol II) is initiated by the hierarchical assembly of the Pre-Initiation Complex onto DNA promoters. Decades of in vitro and yeast research have shown that the TATA-box binding protein (TBP) is essential to Pol II initiation by triggering the binding of other general transcription factors and ensuring proper Pol II loading. Here, we report instead that acute depletion of TBP in mouse embryonic stem cells (mESCs) has no global effect on ongoing Pol II transcription. In contrast, acute TBP depletion severely impairs initiation by RNA Polymerase III. In addition, Pol II transcriptional induction through the heat shock response and retinoic acid differentiation occurs normally in the absence of TBP. Furthermore, we show that the TBP paralog TRF2 binds to gene promoters in TBP-depleted cells, but it does not functionally replace TBP in Pol II transcription. Lastly, we show that the TFIID complex can form and bind to gene promoters with distinct dynamics in TBP-depleted cells and supports Pol II transcription in the absence of TBP.

DISCOVERY OF A POTENT AND SELECTIVE DEGRADER OF THE HISTONE METHYLTRANSFERASE NSD2

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Nuclear receptor-binding SET domain-containing 2 (NSD2) is a methyltransferase responsible for the formation of dimethylated lysine 36 of histone 3 (H3K36me2), a mark generally associated with active transcription. For that, NSD2 plays a vital role in gene regulation, and its dysregulation was reported to be the oncogenic driver in many cancers. Despite efforts to selectively inhibit the catalytic activity of this protein with small molecules, the attempts have been unsuccessful to date. In this presentation, we report the development of a first-in-class NSD2 targeted degrader with sub-micromolar potency that can very selectively reduce the cellular levels of NSD2 protein and the associated H3K36me2 chromatin mark. The degrader contains a simple primary alkylamine warhead that confers proteosome-dependent degradation of NSD2 through a novel E3 recruitment mechanism. Importantly, the degrader mediated degradation of NSD2 results in the down-regulation of pathological phenotypes in multiple myeloma cells, including mild anti-proliferative effects in MM1.S cells containing an activating point mutation and anti-adhesive effects in KMS11 cells harboring the t(4;14)-translocation which up-regulates NSD2 expression. We anticipate this chemical degrader will be a valuable tool for the epigenetics community to investigate NSD2-mediated pathways and their associated therapeutic potentials in cancers.

CHARACTERIZATION OF THE MOLECULAR BASIS OF DNA HYPOMETHYLATION IN RAHMAN SYNDROME: DO GAIN-OF-FUNCTION HISTONE H1 MUTATIONS GLOBALLY DISRUPT DEPOSITION OF H3K36ME2?

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Rahman syndrome (RMNS) is a rare developmental disorder caused by mutations in the H1-4 gene, which encodes the linker histone H1.4 (also known as HIST1H1E). These gain of-function mutations universally yield a truncated H1.4 with a C-terminal Frameshifted Tail (CFT), which in contrast to the wildtype protein has a net negative charge. Fluorescence microscopy of RMNS patient fibroblasts reveals significant alteration of several histone modifications, including reduced H3K9me3 and H3K27me3, resulting in more relaxed chromatin. Several studies have reported a strong correlation between the DNAme profiles of RMNS and two other neurodevelopmental disorders, namely Sotos syndrome and TBRS, caused by loss of function mutations in *NSD1* (which deposits H3K36me2), and the *de novo* methyltransferase *DNMT3A,* respectively. Notably, we and others have shown previously that H3K36me2 guides *DNMT3A*-mediated *de novo* DNA methylation (DNAme). However, despite the similar "episignatures" of these disorders, the underlying molecular basis of the hypomethylation observed in the blood of RMNS patients remains to be determined.
I will investigate whether CFT H1.4 impacts the deposition of histone modifications and in turn DNAme using an *in vitro* differentiation model that recapitulates the widespread *de novo* DNAme observed in early development, namely the naïve mouse

embryonic stem cells (nESC) to epiblast like cells (EpiLC) culture system. Specifically, I will characterize: 1) nESCs in which 9/10 alleles of the somatic H1 genes (a,b,c,d,e) have been deleted ("H1low"), and 2) nESC expressing RMNS/CFT H1.4, to study the impact of such loss-of function and gain of-function mutants, respectively, on the distribution of H3K36me2 and DNAme in EpiLCs.
In preliminary RNA-seq experiments, I have found that expression of tissue specific H1 genes is altered in H1-low mESC (with increased H1t and decreased H1f0 expression, suggesting a feedback mechanism of expression in the H1 family. Furthermore, I observed dysregulation of *Suz12* and *Ezh2*, components of the PRC2 complex and reduced level of H3K27me3 by Western blot. I am currently carrying out ChIPseq to determine whether H3K36me2, is altered in H1-low ESCs and EpiLCs. If changes are observed, I will perform DNAme analysis of H1-low and control EpiLCs.In future experiments, I will introduce an expression vector encoding WT H1.4 or an RMNS/CFT H1.4 mutant into WT ESCs and carry out similar differentiation experiments, focussing on the genome-wide distribution of H3K36me2 and DNAme in this "gain" of function mutant relative to WT EpiLCs. Taken together, these experiments will help us understand how altered H1 impacts crosstalk between H3K36me2 and DNAme, and thereby provide insight into molecular mechanisms that likely contribute to RMNS.

DISENTANGLING THE ROLES OF NSD1/2/3 IN DEVELOPMENT AND CANCER

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Histone lysine methyltransferases (HMTases) catalyzes the transfer of methyl groups to lysine residues on histone tails. These histone marks deposited by HMTases are involved with either active transcription, such as H3K36me, or repressed transcription, such as H3K27me. The nuclear receptor-binding SET domain (NSD) family of HMTases comprises of NSD1, NSD2 and NSD3. They function to mono- and dimethylate histone H3 on lysine 36 (H3K36). Defects in members of the NSD family have been shown to lead to cancer and developmental disorders.
Previous work by our group and others have found that NSD1 loss of function mutations to be drivers of HPV(-) head and neck squamous cell carcinomas (HNSCC). This class of HNSCC is characterized by reduced intergenic H3K36me2 leading to subsequent spreading of H3K27me3 and DNA hypomethylation. Comparatively, NSD2 has been well-documented with respect to its gain-of-function mutations in blood cancers. In multiple myeloma, for example, t(4;14) translocations results in overexpression of NSD2, increased H3K36me2 and shrinkage of H3K27me3 domains. NSD3, on the other hand, has been shown to interact with BRD4, a transcriptional regulator that plays a vital role in tumorigenesis.
Despite the structural similarities, members of the NSD family appear to play non-redundant roles and associate with different cancers and developmental syndromes. Thus, the purpose of this study is to disentangle the function, localization, and catalytic activity of members of the NSD family, with the overall goal of uncovering insights for therapeutic treatment.
Preliminary data using CRISPR-Cas9 knockout of NSD2 in HNSCC cells and NSD3 in mouse mesenchymal cells suggest that NSD2 and NSD3 play minimal roles in these cells. Their ablation did not reduce H3K36me2 and instead, appears to spread into regions previously devoid of H3K36me2. However, NSD2 overexpression in HNSCC NSD1-knockout cells appears to restore H3K36me2 in regions that were depleted due to loss of NSD1, although it also spreads into regions previously devoid of H3K36me2. Transcriptomic analysis indicates that overexpression of NSD2 in these NSD1-depleted cells restores the expression of most genes close to baseline levels. Overall, these preliminary results suggest that NSD2 overexpression can restore the transcriptomic and epigenetic landscape when NSD1 is depleted. Further work will include a doxycycline inducible system with NSD1, NSD2 and NSD3 in the same promoter. We currently have a quadruple knockout of NSD1, NSD2, NSD3 and SETD2KO in a mouse mesenchymal cell line to use with this inducible system. Re-introducing these NSD proteins one at a time to baseline levels in a cell lacking any writers of H3K36me2 will shed light into unique or shared functions among members of the NSD family.

ZMYM2 IS ESSENTIAL FOR METHYLATION OF GERMLINE GENES AND ACTIVE TRANSPOSONS IN EMBRYOGENESIS

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ZMYM2 is a transcriptional repressor whose role in development is largely unexplored. We found that *Zmym2*$^{-/-}$ mice show embryonic lethality by E10.5. Molecular characterization of *Zmym2*$^{-/-}$ embryos revealed two distinct defects. First, they fail to undergo DNA methylation and silencing of germline gene promoters, resulting in widespread upregulation of germline genes. Second, they fail to methylate and silence the evolutionarily youngest and most active LINE element subclasses in mice. *Zmym2*$^{-/-}$ embryos show ubiquitous overexpression of LINE-1 protein as well as aberrant expression of transposon-gene fusion transcripts. Interaction and colocalization data indicate that ZMYM2 homes to germline genes via binding to the non-canonical polycomb complex PRC1.6 and to transposons via the TRIM28 complex. *ZMYM2*$^{-/-}$ human embryonic stem cells also show aberrant upregulation and demethylation of young LINE elements, indicating a conserved role in repression of active transposons. ZMYM2 is thus an important new factor in DNA methylation patterning in early embryogenesis.

THE MECHANISM UNDERLYING THE PIONEER ACTIVITY OF HOX13 FOR THE DIGIT DEVELOPMENT PROGRAM

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The alteration of body plan by the deregulation of Hox genes is extensively studied in different model systems. In mice, the knockout of Hoxa13 and Hoxd13 together (HOX13 hereafter) results in the complete loss of digits and therefore HOX13 is referred to as the master regulator of the digit program. HOX13 transcription factors have recently been shown to function as pioneer factors to modulate the target gene repertoire essential for distal limb/digit specification. During this reprogramming of a subset of mesenchymal cells, HOX13-dependent chromatin accessibility is essential to allow transcriptional activation of the digit-specific genes. What remains unknown is the mechanism underlying this critical function of HOX13. Interestingly, we identified several members of the SWI/SNF complex, an ATP-dependent chromatin remodeler, as potential interacting partners of HOX13. Further, upon conditional inactivation of SWI/SNF complex catalytic subunit, BRG1 (SMARCA4) in the distal limb, we see the loss of digits and the loss of expression of HOX13 pioneer activity-dependent targets. We also observed by ATAC-seq that 75% of the HOX13 dependent pioneer sites had reduced accessibility in Brg1 mutant distal limb. Based on these findings, our working hypothesis is that HOX13 binding to inaccessible chromatin leads to the recruitment of the SWI/SNF complex, which in turn mediates chromatin opening. We are currently testing this hypothesis.
While there are many studies focused on characterizing the chromatin binding activity of pioneer transcription factors in vitro, the mechanisms by which individual pioneer transcription factors shape chromatin structure **in vivo** remain poorly understood, especially in the context of embryonic development. Therefore, this study addresses the fundamental gap in our understanding of the mechanism adopted by the pioneer factor HOX13 to remodel chromatin. Finally, we are currently studying human point mutations in HOX13 known to result in severe digit malformation, without impaired HOX13 binding to chromatin. We hypothesize that these mutations may disrupt HOX13 pioneer activity which in turn impairs digit development. This would not only help us understand the associated human diseases but could allow us to identify amino acids in HOX13 transcription factors that are key to HOX13 pioneer activity.

PATERNAL OBESITY ALTERS THE SPERM EPIGENOME AND IS ASSOCIATED WITH CHANGES IN THE PLACENTAL TRANSCRIPTOME AND CELLULAR COMPOSITION

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Paternal obesity has been implicated in adult-onset metabolic disease in offspring. However, the molecular mechanisms driving these paternal effects and the developmental processes involved remain poorly understood. One underexplored possibility is the role of paternally driven gene expression in placenta function. To address this, we investigated paternal high-fat diet-induced obesity in relation to sperm epigenetic signatures, the placenta transcriptome and cellular composition. C57BL6/J males were fed either a control or high-fat diet for 10 weeks beginning at 6 weeks of age. Males were timed-mated with control-fed C57BL6/J females to generate pregnancies, followed by collection of sperm, and placentas at embryonic day (E)14.5. Chromatin immunoprecipitation targeting histone H3 lysine 4 tri-methylation (H3K4me3) followed by sequencing (ChIP-seq) was performed on sperm to define obesity-associated changes in enrichment. Paternal obesity corresponded with altered sperm H3K4me3 enrichment at imprinted genes, and at promoters of genes involved in metabolism and development. Notably, sperm altered H3K4me3 was localized to placental enhancers and genes implicated in placental development and function. Bulk RNA-sequencing on placentas detected paternal obesity-induced sex-specific changes in gene expression associated with hypoxic processes such as angiogenesis, nutrient transport and imprinted genes. Paternal obesity was also linked to placenta development; specifically, a deconvolution analysis revealed altered trophoblast cell lineage specification. These findings implicate paternal obesity-effects on placenta development and function as one mechanism underlying offspring metabolic disease.

THE ROLE OF TET DNA DEMETHYLASES IN GLIOMA CANCERS WITH HYPOMETHYLATED PHENOTYPE.

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The single allele histone mutation H3.3K27M, present in roughly 50% of paediatric high-grade gliomas (pHGGs) is associated with significant DNA hypomethylation near differentially expressed genes relative to wild-type. This difference in DNA methylation is thought to play an important role in the epigenetic dysregulation that occurs in these cancers. A therapeutic possibility worth pursuing is compensation for DNA hypomethylation by inhibiting DNA demethylase activity using a drug. We aim to study the function of TET family DNA demethylases: TET1, TET2, TET3, in the context of H3.3K27M and H3.3K27M “repaired” cell lines (where the mutant histone H3.3 allele is knocked out using CRISPR), searching for a minimal perturbation that returns H3.3K27M hypomethylated sites to normal. We begin with TET1, TET2, TET3 triple knockout cells and determine, using a whole

genome bisulfite assay, the extent to which DNA methylation levels are restored in the extreme TET-less scenario. We plan to use CRISPR technology to knock-out each TET separately in isogenic clones and measure differences in DNA methylation, defining the regions where each TET specifically acts. We can then compare against similar TET knock-out experiments done in human embryonic stem cells. In short, this work will help assess if chemical inhibition of TET DNA demethylases holds promise as a pHGG cancer therapy, and if so, specificly which TET should be the drug inhibition target.

INVESTIGATING THE ROLE OF EZH2 ACTIVITY IN THE METABOLIC REPROGRAMMING OF TRIPLE NEGATIVE BREAST CANCERS

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Problem: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype treated with chemotherapy and characterized by a high incidence of treatment resistance. Changes in cellular identity due to epigenetic reprogramming and metabolic adaptations of cancer cells contribute to TNBC progression. We previously demonstrated that the methyltransferase Enhancer of Zest Homologue 2 (EZH2), which regulates the trimethylation of lysine 27 on histone 3 (H3K27me3) plays an essential role in TNBC chemoresistance. Our hypothesis is that epigenetic changes resulting from EZH2 activity impact the metabolic profiles of TNBCs and contribute to tumor progression. Our goals are to identify the metabolic adaptations resulting from EZH2 inhibition and to pharmacologically target novel vulnerabilities arising from these adaptations in order to block the progression of TNBCs.
Methods and results: We first identified that the inhibition of EZH2 induces changes in transcriptomic profiles (RNA-seq) and histone modifications (H3K27me3 vs H3K27ac) associated with genes regulating glucose and glutamine metabolism in TNBC models. Subsequently, we observed that EZH2 inhibition differentially impacts the proliferation and viability of TNBC models upon distinct metabolic contexts. We next determined how these metabolic pathways are reprogrammed in response to the pharmacological inhibition of EZH2, using stable isotope tracing of 13C5-glutamine or 13C6-glucose by gas or liquid chromatography and mass spectrometry (GC/MS, LC/MS) and how inhibition of EZH2 modulates TNBC bioenergetic capacities. Finally, our results identified novel metabolic vulnerabilities resulting from EZH2 inhibition in TNBC models.
Conclusion: The results from this research will provide a mechanistic understanding of the regulation of TNBC metabolic profiles by EZH2 and of its role in the proliferation and progression of TNBC. These results will also allow to identify potential metabolic pathways that could be pharmacologically targeted to sensitize TNBC models to EZH2 inhibition and block TNBC growth and survival.

THE NIH COMMON FUND HUMAN BIOMOLECULAR ATLAS PROGRAM (HUBMAP): BUILDING A FRAMEWORK FOR MAPPING THE HUMAN BODY

Dena Procaccini
NIH

NIH Common Fund programs address emerging scientific opportunities and pressing challenges in biomedical research that no single NIH Institute or Center can address on its own but are of high priority for the NIH as a whole. Common Fund programs are short-term, goal-driven strategic investments, with deliverables intended to catalyze research across multiple biomedical research disciplines. Common Fund programs are intended to change paradigms, develop innovative tools and technologies, and/or provide fundamental foundations for research that can be used by the broad biomedical research community.
Knowing how tissue organization influences a cell's molecular state, interactions, and history is critical for enhancing our awareness of variation in organ function across the lifespan and health disease continuum. HuBMAP's goal is to catalyze the development of a framework for mapping the human body at single-cell resolution. The program will run from 2017 to 2025 and involves more than 350 investigators working on the integration of multiomics and molecular imaging technologies that span wide spatial scales and cover a broad range of molecular classes.

INTERACTIONS OF C2H2 ZINC FINGER PROTEINS REVEAL PRINCIPLES OF CHROMATIN ORGANIZATION IN 3D SPACE

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With over 700 members, C2H2-zinc finger proteins (C2H2-ZFPs) constitute the largest, yet least studied, family of human DNA-binding proteins. To better understand their function, we have used affinity purification coupled to mass spectrometry to identify protein-protein interactions (PPIs) for 345 C2H2-ZFPs. From this dataset, we found that, in addition to interacting with cofactors involved in transcriptional regulation, C2H2-ZFPs also interact with proteins involved in other types of biological processes, suggesting that they are multifunctional. Furthermore, we found that C2H2-ZFPs interact extensively with each other, an observation which we confirmed and extended with the binary protein-protein interaction assay LUMIER, with which we identified 1732 PPIs among 204 C2H2-ZFPs. By integrating the PPIs of C2H2-ZFPs with ChIP-seq data and long range interactions of chromatin (LRIs), we show that interacting C2H2-ZFP pairs, as well as interacting C2H2-ZFP-cofactor pairs, have a higher degree of overlap for binding at opposite ends of LRIs compared to negative controls. Furthermore, multiple interacting pairs of such proteins cluster at opposite ends of the same LRIs.

This leads us to propose a model wherein multiple C2H2-ZFPs cooperate to help form LRIs and organize chromatin in 3D space.

CHROMATIN AND TISSUE MECHANICS IN MAMMARY DEVELOPMENT AND CANCER

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The driver of mammary gland development is a multicellular structure called the terminal end bud (TEB), which facilitates invasion, tubulogenesis, and branching to form the mature gland. Cells in the TEB change their shape, location, and mobility in ways that impose mechanical constraints. In this context, chromatin has a major role and can undergo softening in response to mechanical queues. Specifically, chromatin adopts a gel-like state whose stiffness depends on fiber-fiber and protein interactions, both of which can be influenced by histone post translational modifications (PTMs). These PTMs vary between euchromatin and heterochromatin with the latter being critical to chromatin rigidity, reflecting a prominent role for pericentromeric heterochromatin. Pericentromeres are essential for genome integrity and harbor the repressive histone modification H4K20me3 (trimethylated lysine-20 on histone H4). Our previous work has shown a marked reduction of H4K20me3 during breast cancer progression. To understand the drivers and consequence of H4K20me3 changes, we have characterized pericentromeric heterochromatin dynamics in 3D mammary cultures, which recapitulate features of breast cancer progression and invasion. For our studies, we utilized the mouse mammary epithelial cell line NMuMG, which provides a robust platform for imaging and morphological analyses because pericentromeric heterochromatin is organized into larger structures called chromocenters. In 3D culture, NMuMG cells form organoids that undergo tubulogenesis to form subcellular or multicellular protrusions that invade through the surrounding extracellular matrix. Critically, we found that H4K20me3 levels were markedly reduced in cells at the invasive front of individual tubules. Importantly, the invading cells within the tubule are subjected to a range of mechanical forces associated with pushing, pulling, and migration, suggesting H4K20me3 loss may facilitate heterochromatin softening to drive breast cancer phenotypes. Overall, these observations provide a framework for pursuing how H4K20me3 loss affects chromatin rigidity and its contribution to breast cancer pathogenesis.

CANCER CELL TRANSCRIPTOMIC, EPIGENOMIC AND PHARMACOLOGICAL CHANGES IN 3D CULTURE

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Lung cancer (mostly non-small cell lung cancer (NSCLC)) kills about 1.6 million people worldwide each year. Despite advancements in our understanding of NSCLC biology, overall survival remains low, especially once the tumour has spread. Frustratingly, many potential NSCLC drugs work in the laboratory but fail in clinical trials, and we urgently need better experimental models to discover more potent drugs. Three-dimensional (3D) cell culture produces dense clumps of cancer cells called spheroids. Their use can potentially improve drug discovery, as they display several qualities, such as high cell density, a lack of oxygen and nutrients, and the production of a complicated matrix between the cells, that make them better resemble actual tumours. 3D spheroids of immortalized cancer cell lines could be game-changing models in which to study tumour biology and perform screens to identify anticancer drugs; however, the optimal procedures for making and using them as anticancer models remain unknown for most cancers. We have developed a novel “4D” spheroid model that replicates several important features observed in advanced lung cancer that are missing in 2D cells and 3D spheroids. 4D spheroids were exposed to a library of 181 drugs. The type I protein arginine methyltransferase (PRMT) inhibitor MS023 was preferentially cytotoxic to 4D spheroid cells compared to cells grown in 2D. To uncover additional epigenetic vulnerabilities, we performed a combinatorial drug screen using pre-treatment with MS023 followed by the 181 epigenetic drugs. We detected a synergistic interaction between MS023 and JIB-4, a lysine demethylase 4 (KDM4) inhibitor, in 4D spheroids but not in 2D cells. This work will provide a solid preclinical foundation to investigate these drugs as a novel NSCLC treatment. Our 4D spheroid model can also be adapted to other cancer cell lines, providing highly representative models that could be used to find better treatments for a variety of cancers.

EPIGENETIC REGULATORY MECHANISMS OF NEURONAL CELL SPECIFICATION BY CHROMATIN REMODELING PROTEINS

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Cell-type-specific gene expression is established by the interplay between enhancer DNA and chromatin regulatory proteins such as chromatin remodeling complexes. Several subunits of the BAF chromatin remodeling complexes are specifically expressed during the nervous system development in mammals. How neuron-specific chromatin remodeling factors recognize and activate cell-type-specific enhancers in the nervous system remains unknown. Using mouse embryonic stem cell differentiation systems, we show genome-wide mechanisms of how chromatin remodeling factors control specification and maintenance of neuronal cell identities. We reveal that the neuronal BAF subunits contribute to establishment of the enhancers linked to synaptic genes in spinal motor neurons and GABAergic neurons. These enhancer activities correlate with expression of their target genes and accessibility of enhancer DNA, potentially bound by neuronal transcription factors. The knockout of neuronal BAF subunits results in a decreased cell-cycle exit and reduced differentiation rate, promoting the transition from neural progenitors to postmitotic neurons. We also demonstrate that neuronal BAF knockout cells impair embryonic stem cell differentiation into excitatory and inhibitory neurons by repressing neuronal gene expression and dendritic growth. Our results suggest that neuronal gene expression programs are regulated by neuronal enhancers established by cell-type-specific chromatin remodeling complexes.

ESEARCH3D: PROPAGATING GENE EXPRESSION IN CHROMATIN NETWORKS TO ILLUMINATE ACTIVE ENHANCERS

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Most cell type-specific genes are regulated by the interaction of enhancers with their promoters. The identification of enhancers is not trivial as enhancers are diverse in their characteristics and dynamic in their interaction partners. We present Esearch3D, a new approach that leverages network theory approaches to identify active enhancers. Our work is based on the fact that enhancers act as a source of regulatory information to increase the rate of transcription of their target genes and that the flow of this information is mediated by the folding of chromatin in the three-dimensional (3D) nuclear space between the enhancer and the target gene promoter. Esearch3D reverse engineers this flow of information to calculate the likelihood of enhancer activity in intergenic regions by propagating the transcription levels of genes across 3D-genome networks. Regions predicted to have high enhancer activity are shown to be enriched in annotations indicative of enhancer activity. These include: enhancer-associated histone marks, bi-directional CAGE-seq, STARR-seq, P300, RNA polymerase II, and expression quantitative trait loci (eQTL). Esearch3D leverages the relationship between chromatin architecture and transcription, allowing to predict active enhancers and understand the complex underpinnings of regulatory networks. The method is available at: https://github.com/InfOmics/Esearch3D.
A preprint about this work is also available:
https://www.biorxiv.org/content/10.1101/2022.08.04.502774v1

MECHANISMS GOVERNING THE ACCESSIBILITY OF DNA DAMAGE PROTEINS TO CONSTITUTIVE HETEROCHROMATIN

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Chromatin is divided into euchromatin and heterochromatin, with heterochromatin being restrictive to transcription. Heterochromatic regions of the genome have a higher density of chromatin resulting from compaction of the chromatin fibre. One mechanism proposed to regulate the access of molecules to heterochromatin is size-dependent accessibility. Several studies have shown that above approximately 10 nm in diameter, proteins and protein complexes are attenuated in their access to heterochromatin. However, recent work has shown that heterochromatic regions may exist in association with phase separated liquid condensates. This implicates a mechanism where partitioning between the nucleoplasm or heterochromatin is based on relative solubility. We assessed the constitutive heterochromatin concentration of several fluorescently tagged DNA damage response proteins relative to their nucleoplasmic concentration in living mouse cells. Constitutive heterochromatin in mouse cells is organized into large micron-sized chromocenters. We found that proteins above 10 nm in diameter can access chromocenters. Among the sensor proteins, Ku70 and PARP1 demonstrate enrichment within chromocenters. MRE11 exhibited a primarily homogenous concentration between chromocenters and the nucleoplasm, but concentration ranged from depleted to enriched. This variability was found to be independent of recruitment by poly(ADP)ribosylation. While the largest downstream proteins we tested, BRCA1, 53BP1, MDC1, and ATM, were found to be commonly depleted from chromocenters, the lowest mean relative concentration was approximately 50% and all had a large range of concentrations. We also found some smaller downstream proteins, EGFP, and low molecular weight fluorescent dyes are depleted from chromocenters, arguing against exclusively size-dependent regulation. Consistent with this interpretation, we demonstrate that purified GFP has a significantly higher chromocenter concentration in fixed cells than EGFP in live cells. Further, the relative chromocenter concentration of fluorescent dextrans is independent of their molecular weight. Our results demonstrate that even large biomolecules readily access the interior of chromocenters. Instead, relative solubility in the distinct solvent environments of the nucleoplasm and the chromocenter may be an important size-independent influence on the steady-state concentration of each molecule within the chromocenter.

KMT2C HAPLOINSUFFICIENCY RESULTS IN A NEURODEVELOPMENTAL DISORDER WITH DISTINCT CLINICAL FEATURES AND A UNIQUE DNA METHYLATION SIGNATURE

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Introduction. KMT2C is an important epigenetic regulator which acts in KMT2C-COMPASS-like complex, together with KMT2D, to mono- and trimethylate histone 3 lysine 4 (H3K4). *KMT2C* has multiple transcripts, encoding canonical and tissue-specific isoforms. Haploinsufficiency of *KMT2C* results in a syndromic neurodevelopmental disorder (NDD) called Kleefstra syndrome type 2. In contrast, *KMT2D* haploinsufficiency results in the clinically distinct Kabuki syndrome type 1, while Kleefstra syndrome is caused by *EHMT1* haploinsufficiency. As information about the KMT2C-related NDD is limited, we sought to clinically and molecularly characterize it and also discriminate it from Kabuki and Kleefstra syndromes.
Methods. We collected clinical data for 76 cases with pathogenic *KMT2C* variants, as well as 13 cases with *KMT2C* variants of

uncertain significance (VUS). Additionally, blood-derived DNAs from 15 cases with *KMT2C* variants (12 pathogenic and 3 VUS) and from 29 age- and sex-matched controls were available for analysis on the Illumina MethylationEPIC array. Using the DNA methylation (DNAm) analysis pipeline developed in the Weksberg lab, we identified a *KMT2C*-specific DNAm signature, which was tested for classifying *KMT2C* VUS. We also compared the *KMT2C* signature confirmed Kabuki type 1 and Kleefstra syndromes, respectively cases.

Results. Clinical evaluation of cases with pathogenic *KMT2C* variants demonstrated highly variable phenotypes with dysmorphic features, NDD and/or several congenital anomalies. The clinical spectrum, and facial features were found to be distinct from Kabuki type 1 and Kleefstra syndromes. While most cases had *de novo* variants, 15% of cases inherited a pathogenic *KMT2C* variant from a mildly affected parent.

Differential methylation analysis identified a highly specific and sensitive *KMT2C* DNAm signature consisting of 83 DMPs at $|\Delta\beta| > 0.1$ and $q < 0.05$. Most of the significant probes were enriched in promoter CpG islands, which supports a previously reported role of KMT2C in transcription regulation at promoters. Importantly, all tested truncating variants were classified positively, regardless of whether the variant affected only the largest transcript or only brain-specific isoforms. For 3 cases with *KMT2C* VUS, two were classified as controls and one as *KMT2C* positive. These results correlated positively with the clinical assessments, so the variants were reclassified as benign and pathogenic, respectively. Confirmed Kabuki type 1 and Kleefstra syndrome cases did not classify positively on the *KMT2C* signature, which paralleled their distinct clinical features when compared to KMT2C related NDD.

Conclusions. We described a large clinical cohort of *KMT2C*-related NDD and its specific DNA methylation signature, which was useful for defining the condition, classifying *KMT2C* variants, and distinguishing this syndrome from other two related NDDs such as Kabuki type 1 and Kleefstra syndromes.

INVESTIGATING THE ROLE OF BAF53B IN MOUSE NEURONAL GENE EXPRESSION AND AUTISM BEHAVIOURS

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Autism spectrum disorder (ASD) is one of the most prevalent neurodevelopmental disorders, affecting 1 in 66 children in Canada. ASD is characterized by impairments in social communication, restricted interests, and repetitive behaviours. It is well established that ASD is heritable, however, there are hundreds of genes that have been implicated. Chromatin remodeling complexes are attractive candidates to study as they can alter the expression of many genes. The BAF (Brg1 associated factor) complex is one such chromatin remodeling complex, where 12 of the 29 subunit genes that compose the complex have been connected to ASD. Despite this, it is still unclear how mutations in the BAF complex actually lead to ASD. The neuron specific nBAF complex has emerged as a promising candidate as it is thought to coordinate the expression of synaptic genes during brain development. Deletion of mouse Baf53b, one of the neuronal specific subunits of the nBAF complex, disrupts dendritic spine development and results in fewer synapses *in vitro*. Deletion of one or both copies of Baf53b in mice severely impacts synaptic plasticity and long-term memory, and results in social impairments and increased repetitive behaviors. Recently, *BAF53B* was identified as the most significantly mutated gene in the Simons Recessive Autism Cohort. Baf53b is expressed across all neuronal subtypes, but nothing is known about the specific role of Baf53b in interneurons. Specifically, parvalbumin (PV) interneurons are depleted in ASD and mice with reduced levels of PV expression display ASD phenotypes. I hypothesize that conditional deletion of Baf53b in PV neurons will alter gene expression required for PV neuronal function, resulting in PV neuron hypoactivity and ASD-relevant behaviours. A mouse primary neuron culture system was first established to gain insight into Baf53b function. Cortical neuron cultures were created from Baf53b-flox mice at embryonic day 18. Baf53b was deleted following delivery of Cre recombinase with an adeno-associated virus. At 7 days *in vitro* (DIV), there is a significant reduction in dendritic branching following Baf53b deletion when compared to control ($p < 0.0001$, two-way ANOVA). RNA from cultured neurons was sent for RNA-sequencing to determine if there are any nBAF targets that might be responsible for this branching defect following Baf53b deletion. To elucidate the role of Baf53b specifically in PV interneurons, Baf53b will be selectively deleted in PV neurons by breeding mice carrying the Baf53b-floxed allele to mice containing Cre recombinase driven by the PV promoter. The extent of Baf53b deletion within PV neurons will be evaluated in each animal by immunohistochemistry for PV and Baf53b. Both male and female juvenile Baf53bPV-KO, Baf53bPV-Het and wild type littermates will be evaluated for developmental milestones and ultrasonic vocalizations. As young adults these same animals will be assessed for activity, anxiety, memory, social interaction and repetitive behaviours, all of which are relevant to ASD. Evidence is mounting to suggest that hypofunction of PV neurons is fundamental to the pathogenesis of ASD. The proposed research represents a unique interface between neuroscience and computational biology for understanding gene expression in ASD. Identified gene targets can be used for future rescue experiments to test the casual contribution of key regulators and for potential therapeutic development.

REGULATION OF ZNF217 ONCOGENE BY A CO-AMPLIFIED 20Q13 SUPER-ENHANCER REGION IN GASTRIC AND BREAST CANCER

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The chromosome 20q13 region is recurrently amplified in many different cancer types, including breast and stomach cancer. This genomic region harbors the oncogenic zinc-finger transcription factor ZNF217, whose overexpression is associated with the immortalization of cancer cells, metastasis phenotypes, and poor patient prognosis. However, whether additional mechanisms, other than increased copy number, are responsible for ZNF217

hyperactivation remain to be elucidated. Several emerging paradigms suggest that copy number variants (CNVs) target regulatory non-coding regions, either on their own, or together with oncogenes. By analyzing publicly available array data from the The Cancer Genome Atlas (TCGA), we discovered a significantly co-amplified noncoding region adjacent to ZNF217 across 1075 breast and 438 gastric cancer patients that harbors multiple active enhancer elements. To assess the importance of these co-selected enhancers in ZNF217 hyperactivation, we use HiChIP data from several breast and gastric cell lines to prioritize enhancers interacting with the ZNF217 promoter and employ CRISPRi to assess the contribution of these enhancers to ZNF217 hyperactivation. ZNF217 is currently undruggable, therefore identifying the mechanism(s) behind its hyperactivation, such as a dependency on specific regulatory elements, may lead to alternative therapeutic interventions for 20q13 amplified cancers.

ZMYM2 IS ESSENTIAL FOR METHYLATION OF GERMLINE GENES AND ACTIVE TRANSPOSONS IN EMBRYOGENESIS

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ZMYM2 is a transcriptional repressor whose role in development is largely unexplored. We found that Zmym2$^{-/-}$ mice show embryonic lethality by E10.5. Molecular characterization of Zmym2$^{-/-}$ embryos revealed two distinct defects. First, they fail to undergo DNA methylation and silencing of germline gene promoters, resulting in widespread upregulation of germline genes. Second, they fail to methylate and silence the evolutionarily youngest and most active LINE element subclasses in mice. Zmym2$^{-/-}$ embryos show ubiquitous overexpression of LINE-1 protein as well as aberrant expression of transposon-gene fusion transcripts. Interaction and colocalization data indicate that ZMYM2 homes to germline genes via binding to the non-canonical polycomb complex PRC1.6 and to transposons via the TRIM28 complex. ZMYM2$^{-/-}$ human embryonic stem cells also show aberrant upregulation and demethylation of young LINE elements, indicating a conserved role in repression of active transposons. ZMYM2 is thus an important new factor in DNA methylation patterning in early embryogenesis.

CONSERVED ROLE OF THE LNCRNA *CRNDE* IN REGULATING SENESCENCE AND PROMOTING COLORECTAL CANCER CELL PROLIFERATION.

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The lncRNA *Colorectal Neoplasia Differentially Expressed* (*CRNDE*) has been shown to be upregulated in pre-cancerous colon adenomas and colorectal adenocarcinomas (CRC). Its overexpression also correlates with disease stage progression and poor prognosis. While *CRNDE* has been reported to be an oncogene in multiple other malignancies, its mechanism of action remains poorly characterized. Here, we generated both human cellular and *in vivo* mouse gain- and loss-of-function models to further characterize the role of *CRNDE* in tumorigenesis. Using the CRISPR-Cas9 system, we ablated the genomic region containing *CRNDE* in HCT116 (P53$^{+/+}$), a human colorectal cancer cell line expressing high levels of *CRNDE*. We also derived primary embryonic fibroblasts (MEF) from transgenic mice overexpressing the murine form of *Crnde*.
We found that both overexpression of *Crnde* in primary untransformed MEFs leads to increased proliferation and enables the bypass of replication-induced senescence, as shown by decreased b-galactosidase activity and reduced expression of the senescence markers p21^{Cip1} and p16^{INK4a}. Conversely, genetic deletion of the *CRNDE* locus in human colorectal cancer cell line HCT116 (p53$^{+/+}$) results in decreased cell proliferation and increased senescence following doxorubicin-induced DNA damage. These effects are rescued *in trans* by ectopic re-expression of all 4 human as well as 2 mouse *CRNDE* isoforms, indicating conserved function. Cell fractionation followed by RT-qPCR revealed that both human and mouse *CRNDE* are predominantly bound to chromatin. To identify the proteins associated with *CRNDE* in HCT116 cancer cells, we performed ChIRP-MS and found several proteins involved in telomere maintenance and DNA damage response, such as TRF2 and RAP1 part of the shelterin complex, TP53RK as well as MDC1.
Together, these data suggest that *CRNDE* can promote tumorigenesis by regulating cell proliferation and senescence and that this conserved function may be related to its ability to bind chromatin and interact with proteins involved in telomere maintenance as well as DNA damage response. *In vivo* animal models and structure-function analyses are currently being performed to further understand the oncogenic properties of this lncRNA.

CHARACTERIZATION AND THERAPEUTIC TARGETING OF THE HISTONE LYSINE DEMETHYLASE KDM4A IN ACUTE MYELOID LEUKEMIA

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Epigenetic modifications modulate gene expression, genome stability and nuclear architecture. Research aimed at better understanding the implications of epigenetic differences in cancer cells is a promising strategy in the development of novel therapies and clinical care. We determined that KDM4A, an epigenetic regulator of the family of JUMONJI lysine demethylases, is overexpressed in tissue samples from patients with MLL-AF9 pediatric acute myeloid leukemia (AML) and is required for leukemic cell proliferation. Using molecular and pharmacological inhibition methods, we have demonstrated that the targeting of KDM4A in MLL-AF9 AML cell lines leads to a cellular differentiation phenotype, a decreased expression of genes involved in leukemic maintenance and increased expression of cellular senescence markers. At transcriptional start sites and genomic enhancer regions of key MLL-AF9 AML oncogenes, we observed an increase in levels of chromatin-repressive H3K9me3 upon KDM4A depletion or inhibition and decreased levels of H3K27ac, a marker of accessible chromatin. We have also performed in vitro and in vivo experiments to demonstrate that pharmacological inhibition of KDM4A in combination with BH3-mimetic drugs may represent an effective strategy for enhanced elimination of leukemic cells. Our study will decipher a precise molecular role of KDM4A in the maintenance of the leukemic proliferation transcriptional program and describe a potential therapeutic approach for treatment of MLL-AF9 AML, a disease still plagued with dismal survival rates.

ANALYSIS OF DONOR VARIABILITY IN NORMAL ADULT FEMALE MAMMARY GLAND CELL METHYLOMES

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The mammary epithelium is composed of a basal myoepithelial cell layer (BCs) and a luminal layer made up of estrogen receptor-expressing cells (termed luminal cells or LCs) and milk lineage cells (termed luminal progenitors, LPs). Previous studies from our group have described the epigenomic differences between these 3 lineages of mammary epithelial cells and breast stromal cells (SCs) derived from premenopausal women (< 45 years of age); however, the epigenomic changes that occur in these 4 cell types during menopause is not known. A direct comparison of cells purified from tissues obtained from pre- and postmenopausal women would enable such a comparison; however, the low epithelial cellularity in postmenopausal breast tissue makes the purification of the number of cells required for the analysis impractical. To circumvent this problem, we propose to use pooled breast cell samples from multiple postmenopausal donors. To validate that pooled samples, have epigenomic profiles that are representative of profiles derived from individual donors, we made use of 2 existing groups of epigenomic datasets previously generated from the 3 types of mammary cells obtained from premenopausal women, along with surrounding stromal cells. We hypothesize that epigenomic measurements derived from pools of purified cell types provide a quantitative representation of cell type and enable comparative analysis. To test this hypothesis whole genome bisulphite sequencing was performed on genomic DNA isolated from a biological pool of 6 individuals plus and 7 parallel datasets were generated on the same cell types isolated and analyzed individually from 7 premenopausal subjects. To compare the outputs of pooled vs. individual samples, fractional methylation calls were generated for the ~26 million CpG sites present in the GRCh38 build of the human reference genome. A pairwise differential methylation analysis using a Benjamini-Hochberg corrected Fisher's exact test between individuals and between the pool and individuals was performed to identify significantly differentially methylated CpGs (DMCs; FDR <0.01 and >25% fractional methylation change). A significant reduction in the number of DMCs between individual and pooled measurements compared to individual vs. individual comparisons was observed across the 4 breast cell types. This analysis suggests that epigenetic measurements derived from the 6 pooled samples provide a more accurate reflection of the average epigenomic state of normal premenopausal cells compared to measurements derived from any single individual. Because of this, future studies of postmenopausal breast tissue will rely on pools of cells derived from multiple donors.

PREDICTING CRISPR-CAS9 OFF-TARGET EFFECTS IN HUMAN PRIMARY CELLS USING BIDIRECTIONAL LSTM WITH BERT EMBEDDING

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Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system is a ground-breaking genome editing tool which has revolutionized the cell and gene therapies. One of the essential components involved in this system ensures its success is the design of an optimal single guide RNA (sgRNA) with high on-target cleavage efficiency and low off-target effects. This is challenging as many conditions need to be considered, and empirically testing every design is time-consuming and costing. *In silico* prediction using machine learning models provide high performance alternative opportunities. In this study, we present CrisprBERT, a deep learning model incorporating a BERT architecture for embedding paired sgRNA, DNA sequences and bidirectional LSTM for learning, to predict the off-target effects of sgRNAs through utilizing the sgRNAs and their paired DNA sequences only. We propose a new doublet stack encoding to capture the local energy configuration of Cas9 binding and applied BERT to learn the better embedding of the doublet pairs. Our model achieved better performance than several state-of-the-art deep learning models, including DeepCRISPR, CRISPR-Net and AttnToMismatch_CNN, regarding 10-fold and leave-one-sgRNA-out cross-validations as well as independent testing.

DECONSTRUCTING THE H3K36ME2 NETWORK IN CANCER AND DEVELOPMENT - FROM REGULATORY POTENTIAL TO THERAPEUTICS

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The epigenetic state of a cell plays a critical role in mediating the interactional network of transcription factors and their target genes, primarily by influencing chromatin accessibility. Histone 3 lysine 36 dimethylation (H3K36me2) is a histone post-translational modification associated with active and regulatory regions of the genome. It is broadly distributed within intergenic regions by the lysine methyltransferase NSD1, it acts as a binding site for DNMT3A to deposit *de novo* DNA methylation (DNAme), and it has an antagonistic relationship with the PRC2-mediated repressive mark H3K27me3, whereby depletion of H3K36me2 is often accompanied by spreading of H3K27me3 and vice versa. Previous work by our group and others, has shown that the loss of NSD1 found in a subgroup of HPV(-) head and neck squamous cell carcinomas (HNSCC) - and corroborated in mouse embryonic stem cells - leads to a loss of intergenic H3K36me2, and drives tumorigenesis through down-regulation of target genes due to a pervasive gain of H3K27me3 and DNA hypomethylation. Comparatively, the effects on depletion of H3K36me2 through ablation of the NSD1 paralogs, NSD2 and NSD3, have primarily been documented in the context of elevated H3K36me2 in cancer cells where they exist as hyperactive variants, or where they are overexpressed due to translocation or amplification events. In multiple myeloma, overexpression of NSD2 and increased H3K36me2 is linked to the gain of new topologically associating domains (TADs) and the strengthening of existing TADs, concordant with increases in CTCF and cohesin binding. In addition to its role in cancer, all NSD3 isoforms have been shown to physically interact with BRD4.
While the spatial and functional coordination of H3K36me2 regulation by the NSD family of proteins remains uncharacterized, genome wide depletion of intergenic H3K36me2 from the loss of NSD1 in HNSCC cells and knockout studies of H3K36me2 'writers' in mouse mesenchymal stem cells (mMSC) exposes a unique opportunity to deconstruct the H3K36me2 network. Specifically, a mMSC NSD1/2-SETD2-TKO cell line was previously generated (hereafter called 10T-TKO), and analyses of ChIP-seq data reveals a global depletion of H3K36me2, however, sparse and narrow peaks remain at specific regulatory elements throughout the genome. The initial hypothesis here is that *NSD3 is responsible for depositing the remaining H3K36me2 in 10T-TKO cells*. This cell line lends itself as a unique system to not only dissect the functional roles of NSD3-mediated H3K36me2, but also to identify target genes regulated by NSD3 catalytic activity which remains largely uncharacterized. Concurrently, ablation of NSD2 in HNSCC cells has shown little change to the quantity of H3K36me2, however, H3K36me2 does appear to spread into genomic regions previously unoccupied by the mark. Overexpression of NSD2 in cells lacking NSD1 appears to restore global H3K36me2, however, similar to NSD2-KO cells, H3K36me2 appears to spread into regions previously unoccupied by the mark. RNA-seq data indicates that overexpression of NSD2 appears to restore the majority of differentially expressed genes in NSD1 loss of function cells. Future work will include overexpression of NSD1 and NSD3, and to further elucidate potential functional redundancy between NSD family members, as well as uncovering potential therapeutic avenues in HNSCC.

INTEGRATING CHROMATIN ACCESSIBILITY DATA WITH THE DATA FROM GENOME-WIDE ASSOCIATION STUDIES UNCOVERS TRANSCRIPTION FACTORS INVOLVED IN NINE AUTOIMMUNE DISEASES

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Autoimmune and inflammatory diseases are a group of > 80 complex diseases caused by loss of tolerance of the immune system for self-antigens. The biological mechanisms of autoimmune diseases are largely unknown, preventing the development of effective treatment options. Integrative analysis of genome-wide association studies and epigenetic data has shown that the risk variants of autoimmune diseases are enriched in epigenetic regions of immune cells, supporting their role in gene regulation. However, we still lack a systematic and unbiased identification of transcription factors (TF) involved in disease gene regulation.
We hypothesized that for some of the disease-relevant TFs, their binding to DNA is affected at multiple genomic sites rather than a single site, and these effects are cell-type specific. In this study, we developed a statistical approach to assess enrichment of TFs in being affected by disease risk variants at multiple sites. We used genetic association data of nine autoimmune diseases (obtained from Immunochip) and identified 99% credible interval (CI) SNPs for each trait. We then integrated CI SNPs and DNase-I footprinting data of 376 samples comprising 35 unique cell types and employed a probabilistic model to identify the CI SNPs that are likely to change binding probability of certain TFs at specific cell types. Finally, for each TF (out of 1,372 TFs), we used Fisher's Exact test to assess whether CI SNPs show enrichments in terms of changing the binding probability of that TF at multiple sites (FDR < 10%).
Our analysis resulted in identification of significantly enriched TFs and their relevant cell types for each trait. The number of prioritized TFs in immune cell types varied between 1 and 14 for seven autoimmune diseases. For the two other traits, we did not find any significant TFs, likely due to a lack of statistical power because of their smaller sample sizes in Immunochip data. Our analysis identified some TFs previously known to be relevant to autoimmune diseases (e.g. Ahr:Arnt for rheumatoid arthritis and SPI-B for multiple sclerosis), and some other less studied new TFs. The enriched cell types also varied across the traits (e.g., CD8 and Mobilized CD4 T cells for rheumatoid arthritis, and CD56 and Mobilized CD4 T cells for multiple sclerosis). Our ChromHMM analysis proved that our predicted DNase-I footprinting sites are active enhancers or promoters in the relevant cell types. Additionally, our GREAT pathway analysis showed that the majority of the significant biological pathways are immune-

related, an example of which is B cell adhesion pathway in multiple sclerosis.
Although we applied our framework to the data from autoimmune diseases, our model is general and can be applied to genetics association data from various common complex diseases. This will identify disease-relevant TFs and their relevant cell types, and will facilitate discovering specific gene regulatory mechanisms of complex diseases.

DECIPHERING EPIGENETIC MEMORY: A NEW TOOL TO TEST ELEMENTS OF MOLECULAR MEMORY

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Polycomb group proteins (PcG) play an essential role in the transmission of cell fate by maintaining gene repression through cellular divisions. How PcG-mediated repression is inherited is still unclear. The histone modifications H3K27me3 and H2AK119ub, catalysed by the PcG complexes PRC2 and PRC1, respectively, and PcG proteins themselves have been suggested to carry epigenetic memory. As molecular memory elements, they are expected to be transmitted through the critical cell cycle events, DNA replication and mitosis. The extent to which transmission of modified histones is important for epigenetic memory is still not well understood.
To determine whether modified histones present at PcG regulated genes carry epigenetic memory, we are developing split versions of the self-labeling HaloTag as a tool to covalently mark histones at PcG protein binding sites, and subsequently track them through cell division.
We identified a new split HaloTag and used it to visualize specific protein-protein interactions, including protein-histone interactions. When each of the two parts of the split HaloTag are fused to a PRC1 subunit, interactions between the subunits are observed. When one part of the HaloTag is fused to a PcG protein, and the second part, containing the labeling site, to Histone H3, chromatin labeling is observed. This configuration will be used to label histones at PcG binding sites using a biotinylated HaloTag substrate. Genomic approaches will be used to track the labelled histone cohort through cell division, to determine whether parental (i.e., modified) histones are retained at their original location.
Thus, our split HaloTag can assess if histone modifications are eligible to carry PcG memory. The split HaloTag could be used to test the memory capacity of other histone modifications and their corresponding regulatory proteins, and more broadly to monitor protein-protein (including protein-histone) interaction histories.

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.
The last mandate for the mapping centre renewals (2017-2022) was the promotion of epigenetic research within Canada by reserving 30% of the funded mapping capacity to cover the cost of profiling samples nominated and provided by the epigenetics research community. This community access program has generated high level of interest and top-ranked projects were selected for further analysis. Three projects have been selected for reference epigenome mapping at the McGill EMC platform, and the processing was completed in 2021. The second phase of the community access program will begin shortly.
Aside from the community samples program, McGill EMC works closely with collaborators on various projects by providing technical and analytical expertise. Furthermore, the recent implementation of Cut&Run and Cut&Tag techniques as an alternative to ChIP-Seq allows us to bonify the offer to collaborators according to histone modification mapping. Hence, these new techniques can provide more flexibility according to the quantity of input material needed and allow for higher throughput by significantly reducing the hands-on time during the processing.
The McGill EMC team is always looking for new challenges and collaborations to serve the Canadian epigenetic community.

EPIGENETIC REWIRING IN TRIPLE-NEGATIVE BREAST CANCER PATHOGENESIS IS RELIANT ON GENOMIC INSTABILITY AND ITS SIGNATURES.

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Epigenetic rewiring plays crucial role in cancer pathogenesis, cancer advancement and cancer drug resistance. This epigenetic

rewiring involves DNA methylation and two major histone modifications, trimethylation & acetylation of histone 3's lysine 27 (H3K27me3 and H3K27ac respectively). Both DNA methylation and histone modifications influence each other, even though different regulators are responsible for these marks. However, to what extend and towards what direction this relationship in Triple-negative breast cancer (TNBC) changes during cancer pathogenesis is not fully understood and the relationship between epigenetic rewiring and mutational background is unclear. Moreover, based on CNA-SV patterns our lab recently defined two major mutational subtypes of TNBC, homologous recombination deficiency (HRD) and fold-back inversion (FBI), with distinct drug resistance characteristics whose epigenomes are not understood at all. Therefore, to answer these questions, TNBC Patient-derived xenograft (PDX) samples encompassing both mutational signatures were used; along with 184hTERT diploid breast epithelial cell lines with wild-type genomic background (WT184hTERT) as epigenetic rewiring control. As HRD mutational instability is known to be dependent on BRCA1 and p53 mutations, therefore, p53-/-BRCA1-/- 184hTERT is used as an additional control for HRD and cancer pathogenesis. DNA methylation at bulk level was identified using Nanopore sequencing, while histone modification landscape was identified based on single cell Cleavage Under Targets & Tagmentation (scCUT&Tag). Compared to non-cancerous WT 184hTERT, DNA methylation landscape in TNBC samples revealed significant anti-correlation with pseudo-bulk histone modification landscape (H3K27ac and H3K27me3) in TNBC. These results were corroborated using the chromatin data from MDA-MB-468 breast cancer line, further confirming the epigenetic rewiring where histone modification and DNA methylation landscape are getting decoupled in cancer cells. Moreover, the reference H3K27ac epigenome landscape for HRD and FBI mutational signatures within heterogenous TNBC PDX was defined at single-cell resolution; both signatures enriched for different biological processes. Very interestingly, these two identified reference epigenomes when compared to DNA methylation from different tumors and p53–/–BRCA1–/– 184hTERT revealed differential anti-correlation dependent on their mutational signatures (HRD or FBI). Hence, illustrating that mutational signature of tumor can be predicted just based on DNA methylation and that epigenetic rewiring depends on corresponding genomic instability signature. Further validation and elucidation of this phenomenon in multiple other TNBC samples and other cancer types with different mutational backgrounds can pinpoint specific structure variants associated with the driver genes, regulators, and transcription factors that are responsible for this cancer pathogenesis and epigenetic rewiring.

CONSERVED GATA4/5/6-MEDIATED GENE REGULATION IN CARDIAC DEVELOPMENT AND DISEASE

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Early cardiac development is orchestrated by a core set of deeply conserved cardiac transcription factors (TFs) and the cis-regulatory elements (CREs) they bind. Mutations in these TFs, or the CREs that regulate them, can lead to congenital heart disease (CHD). However, we still have an incomplete knowledge of the CREs required for normal heart development.
To identify cardiac CREs essential for heart development, we performed a comparative zebrafish-human epigenomic study focussing on the GATA4/5/6 family of TFs. GATA4/5/6 function at or near the top of the cardiac regulatory network hierarchy in animals and are among the earliest TFs expressed in the cardiac mesoderm. We first profiled the open chromatin landscape of gata5-expressing pre-cardiac cells in mid-gastrulation zebrafish embryos. Using a Gata5/6 knockdown, we identified 1,470 differentially accessible regions (DARs) with reduced accessibility upon Gata5/6 loss. Indicative of a direct role of Gata5/6 in establishing chromatin accessibility, these closed DARs showed a strong enrichment for GATA motifs. We identified 47 mesendodermal-specific GATA-dependent DARs as accessible regions conserved between zebrafish and human, which we termed GATA-dependent accessible conserved non-coding elements (GaCNEs). 17 out of 18 GaCNEs tested so far displayed cardiac activity in transgenic zebrafish embryos. Supporting their functional conservation, three GaCNEs were identified as being GATA4 targets in human cardiomyocytes and accessible in cardiac progenitors. Zebrafish deletions of GaCNE1, which forms long range interactions with zebrafish hand2 (130 kb) and human HAND2 (460 kb), resulted in reduced hand2 expression and laterality defects, including cardiac patterning defects. We found the remaining two GaCNEs contain hits for ultra-rare human variants in patients with unsolved congenital heart disease. One such region near TBX20 (GaCNE20) interacts with the TBX20 promoter in the human mesoderm. Both the zebrafish (zGaCNE20) and orthologous human (hGaCNE20) sequences can spatiotemporally recapitulate endogenous zebrafish tbx20 expression. We are currently modelling the ultra-rare variants seen in hGaCNE20 identified from a CHD patient using zebrafish enhancer reporter assays.
Collectively, we identified zebrafish-human conserved GATA-dependent cardiac CREs, which likely contribute to vertebrate cardiac development. Given the proximity of many conserved accessible non-coding elements to dosage sensitive trans-factors, human genetic variation impacting these regions warrants further functional study.

RETROTRANSPOSONS-INDUCED IMMUNE RESPONSES IN CHRONIC INFLAMMATORY DISEASES

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Chronic inflammatory diseases (CIDs) are a group of incurable diseases that exhibit prolonged or recurring inflammation symptoms. These disorders could clinically differ from their target

organ, like Inflammatory Bowel Diseases in the guts, to systemic inflammation, such as Systemic Lupus Erythematosus. Studies have mostly investigated the genetic basis of these diseases, mainly cytokines and immune gene expression. Yet, the transcriptional changes of coding genes cannot fully explain the complications of these diseases. Hence, studies on epigenetic alterations are emerging to understand the underlying mechanisms in immune gene regulation. An important component of gene regulation is promoters and enhancers. Intriguingly, retrotransposons, which are viral remnants contributing to about 40% of the human genome, comprised these regulatory sequences. They could potentially regulate gene transcription as *cis*-regulating elements. However, these elements have largely been ignored in genomic and epigenomic studies due to their repetitive nature. The physiological impact of retrotransposons in inflammatory responses remains largely unknown. To understand the role of retrotransposons in CIDs, we analysed transcriptomic data from a large inflammatory disease cohort in collaboration with SYSCID. Utilising our novel approach to delineate information from retrotransposons, we identified elements that were dysregulated in CIDs associated with immune responses. We also observed disease-specific expression patterns of retrotransposons in various CIDs. Ultimately, this endeavor may lead to the discovery of new biomarkers and futuristic approaches to personalized medicine.

TRANSCRIPTIONAL REGULATION AND CHROMATIN ARCHITECTURE MAINTENANCE ARE DECOUPLED FUNCTIONS AT THE SOX2 LOCUS

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Almost all of the cells in our body contain the same genome sequence, but it is the selective interpretation of these instructions that gives rise to the diversity of cell types and organization into functional tissues. Genes comprise approximately 1-2% of the genome and their coordinated expression governs cell identity and complex behaviours; however, we still do not understand exactly how selective gene expression is orchestrated. Enhancers are a type of regulatory element which, over vast genomic distances, can increase the transcription of one or multiple genes in a cell type-specific manner. Unlike genes or promoters, enhancers currently do not have any known positional or sequence-level features that would permit accurate localization. Although contemporary methods to predict enhancers based on large-scale sequencing experiments find instructive features associated with transcriptionally active chromatin — such as binding by transcription factors, acetylation of histone proteins, or three-dimensional genome configurations that facilitate physical contact between enhancers and genes — they still do not explain why regions with similar enrichment for these marks do not always have similar activity.
We have recently uncovered that transcriptional activation of Sox2 by a strong enhancer can be experimentally decoupled from the ability to form long-range contacts with the Sox2 locus in mouse embryonic stem cells (ESCs), highlighting that these higher-level features are insufficient to understand the mechanisms of enhancer function. Within the canonical Sox2 enhancer are four regions with enrichment for multiple transcription factor binding as well as histone H3K27ac modifications. Using CRISPR-Cas9 mediated deletions, we have identified that the majority of Sox2 expression can be explained by only two of these regulatory regions which rely on a few key transcription factor binding sites. However, using circular chromosome conformation capture followed by sequencing (4C), we observe that local chromatin conformation is widely distributed across multiple transcription factor bound but transcriptionally inert chromosomal locations. This architecture is maintained in a CCCTC binding factor (CTCF) independent manner and is resilient to heavy genomic perturbations and deletions across this locus. Collectively, these data demonstrate the resiliency of this topologically associated domain and highlight that much is still unknown as to what drives long-range enhancer promoter interactions.

A PROXIMAL ENHANCER CLUSTER MAINTAINS SOX2 EXPRESSION DURING EARLY MOUSE NEURAL DIFFERENTIATION

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SRY-box 2 (*Sox2*) is a critical transcription factor for neural stem and progenitor cell (NSPC) commitment during embryonic development in mammals. Studies in the adult mouse brain have shown that *Sox2* expression is required for NSPC self-renewal and differentiation. The importance of SOX2 dosage for human neural development is underscored by observations that patients haploinsufficient for SOX2 present with a spectrum of congenital optic and forebrain defects. During neural differentiation *Sox2* transcription is maintained, but the histone acetylation and chromatin-chromatin interaction profiles surrounding the *Sox2* locus are altered, which indicates that the regulatory landscape undergoes reorganization. However, we do not understand how *Sox2* transcription is maintained throughout neural lineage induction or in the NSPC fate. Using allele-specific deletion, transcriptome analyses, and chromatin accessibility approaches, we investigate a proximal downstream cluster of *Sox2* regulatory regions (SRRs) in a hybrid F1 (*M. musculus/129* × *M. castaneus*) pluripotent stem cell-derived NSPC model.
Using CRISPR-Cas9 mediated deletions, we determined that distal *Sox2* enhancers required for embryonic stem cell pluripotency are dispensable for both neural differentiation and NSPC derivation. We focused on a cluster of putative regulatory elements spanning 2-18 kilobases downstream of *Sox2* (SRR2-18) exhibiting neural lineage-specific transcription factor binding. To understand how *cis*-regulation by SRR2-18 impacts *Sox2* and global gene regulation in the neural lineage, we generated mono- and bi-allelic deletions of SRR2-18. *Sox2* expression profiling during neural lineage induction by allele-specific RT-qPCR showed significant ($P < 0.05$) reductions in Sox2 transcription from the targeted allele compared to the parental line. Bulk RNA-seq analyses of *in vitro* derived NSPCs revealed transcriptomic

perturbations related to neurodevelopmental processes with the loss of SRR2-18. Bulk ATAC-seq with footprinting analysis showed altered chromatin accessibility patterns and over-represented transcription factor motifs in SRR2-18$^{-/-}$ NSPCs which are associated with developmental processes such as neural cell maturation and tissue patterning. These data suggest that the maintenance of *Sox2* transcription during neural differentiation is controlled at the transcriptional level by a downstream proximal cluster of enhancers distinct from previously established *Sox2* regulatory or locus control regions.

EXAMINING THE ROLE OF HISTONE LACTYLATION IN MICROGLIAL INFLAMMATORY RESPONSE

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Microglia, the resident immune cells of the central nervous system, monitor their microenvironment and facilitate innate immune responses when pathogens are encountered. When microglia are activated by a pathogen, they switch to favour a proinflammatory phenotype characterized by a production of proinflammatory cytokines and increased phagocytosis. Previous research has shown that activated microglia undergo a switch to aerobic glycolysis for energy production called the Warburg Effect. Inhibiting this metabolic switch reduces inflammatory responses of microglia. The Warburg effect results in pyruvate being reduced into lactate in the absence of oxidative phosphorylation to result in the regeneration of the oxidation agent NAD+ from its reduced form NADH; this results in high cellular levels of lactate. Recent research from macrophages identified a novel histone mark derived from high levels of lactate (histone lactylation) that impacted the gene expression profile favouring inflammatory cytokine production. We hypothesize that histone lactylation is important for the microglia inflammatory response and that inhibiting lactylation reduces the production of inflammatory cytokines in response to a bacterial derived PAMP, lipopolysaccharide (LPS). Using the BV2 microglial cell line, we assessed how global histone lactylation levels changed in response to LPS stimulus using flow cytometry. We observed that LPS resulted in a global increase of histone lactylation marks. Using sodium oxamate, a potent inhibitor of lactate dehydrogenase A, we determined that the increase of lactylation in response to LPS was attenuated by inhibition of LDHA. We then assessed how inhibiting LDHA would impact the gene expression of pro-inflammatory cytokines in response to LPS and determined that treating with 10mM Sodium Oxamate resulted in a reduction of expression of proinflammatory cytokines including IL1β, TNFα, and Cxcl16. These results suggest that histone lactylation is involved in facilitating the inflammatory response to LPS in microglia. Further research will include examining specific gene loci for lactylation changes and generating a knockout of LDHA in the BV2 cells to eliminate any potential off target effects of sodium oxamate.

EPIGENETIC AGE PREDICTION IN TARGETED METHYLATION SEQUENCING

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DNA methylation levels at specific CpG sites can be used to predict epigenetic age using a mathematical algorithm known as the epigenetic clock. Current epigenetic clocks have been developed using penalized linear regression approaches such as elastic net regression (ELNet) based on methylation arrays. However, there are areas for further improvement of these clocks as arrays cover only a small subset (~3%) of CpGs across the genome, and may omit sites associated with age. Moreover, linear regression approaches do not account for non-linear changes in methylation. Advances in sequencing technology and machine learning models (MLM) show a potential opportunity for the development of novel epigenetic clocks.

Objectives:

1. To find novel age informative CpG sites in methylation sequencing data; 2. To use these sites to develop novel epigenetic clocks using different approaches.

Methods: Targeted bisulfite methylation sequencing (Illumina's TruSeq MethylCapture library) was completed on 932 samples from three Canadian studies: The Canadian Asthma Primary Prevention Study (CAPPS, n=632 samples); the Saguenay-Lac-Saint-Jean study (SLSJ, n=180 samples) and the Canadian Peanut Allergy Registry (CanPAR, n=120 samples). CAPPS is a longitudinal birth cohort that follows children at high-risk for developing asthma from birth to year 15, with methylation sequencing at one to three timepoints: birth, and/or years seven and 15. Maternal samples were also included. SLSJ consists of three-generational triads from families of French-Canadian descent. CanPAR is a registry of individuals at high risk of developing peanut allergy.

Quality Control (QC) such as sex check, principal component analyses and cell counts deconvolution were performed with 914 of the 932 samples remaining in the study. Samples were divided into a "training" set (CAPPS and SLSJ, n=794) and a "validation" set (CanPAR, n=120). Age informative CpGs were identified using linear and mixed effects regression and in training set. Novel epigenetic clocks were developed using elastic net regression and gradient boosting trees (GB) and their accuracy assessed using mean absolute error (MAE, MAE=|predicted age-reported age|).

Linear and mixed effects Regression models identified hundreds of thousands of age informative CpGs (p-value $<1x10^{-8}$) in the Illumina TruSeq library which have not been previously studied. The 1,000 CpG sites with lowest p-values were then used to

develop two novel epigenetic clocks: a 309 CpG ELNet -based clock and a 250 CpG GB-based clock. In the validation set, the GB clock outperformed the ELNet clock (MAE: 1.79 vs 2.64 year). In conclusion, targeted methylation sequencing is a novel technology, which together with advancements in MLMs, provides opportunity for development of more accurate epigenetic clocks.

PROFOUND DIFFERENCES IN BOTH THE METHYLOME AND HYDROXYMETHYLOME OF THE LUNG FIBROBLAST CELLS FOLLOWING ACUTE AND CHRONIC ALPHA IRRADIATIONS TO ALPHA PARTICLES

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It is well-known that the most significant contributor to the average dose of human exposure to natural background sources of radiation in Canada and worldwide, is from alpha-emitting radon gas and its short-lived decay products. Radon is considered the second leading cause of lung cancer after smoking, causing an estimated 16% of deaths per year in Canada according to a Health Canada Report from 2014. Mutations in nuclear DNA induced by alpha-radiation have been well documented, however, our study sheds light on a rarely explored area, epigenotoxic effects induced by alpha particles. Recent studies using both human and animal models, suggest that some epigenetic markers, especially DNA methylation, are strong molecular indicators of health and aging effects associated with environmental stressors, and have the potential to affect many generations. Therefore, this project examines the modifications in the landscape of both the DNA methylation and hydroxymethylation of the genome, two major epigenetic markers, in human lung fibroblast cells irradiated to different doses of alpha particles delivered under an acute and a 14-day chronic treatment. Primary lung fibroblast cells underwent *in vitro* exposure to human-comparable range of doses of ^{241}Am alpha particles at the University of Calgary. We uncovered that acute treatment to alpha particles tends to induce a decrease in both 5-methylcytosines (5mC) and 5-hydroxymethylcytosines (5hmC) in the irradiated fibroblast cells, whereas chronic irradiation was more prone to induce an increase in global methylation and hydroxymethylation levels. We also observed that the increase in 5mC sites following acute treatment was mostly localized in the DNA repeat regions (37%), while the decrease in 5mC sites was mostly detected in the gene bodies (57%). Similarly, both high (68%) and low (40%) levels in 5mC sites induced by chronic treatment to alpha particles were more frequently found at the gene bodies, which could play a role in the development of cancer. Moreover, our findings revealed that the chronic treatment to the particles influences aging, as many candidates of DNA methylation (DNAm) biomarkers of aging were identified in the chronically exposed fibroblast cells. The initial analysis of the epigenetic data that will be presented seems to indicate that chronic alpha particles exposures, compared to acute exposures, has a greater capacity to cause epigenetic events linked to aging and cancers.

HISTONE LYSINE METHYLATION REGULATES THE STABILITY OF ONCOGENE-INDUCED SENESCENCE

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In response to oncogene activation, cells trigger a complex mechanism culminating in cell cycle arrest, known as oncogene-induced senescence (OIS). Hallmarks of cellular senescence include the formation of promyelocytic leukemia (PML) bodies and senescence-associated heterochromatin foci (SAHF), specialized nuclear structures controlling the repression of the E2F-target genes involved in cell proliferation. SAHF are dense chromatin domains enriched in transcriptional repressive marks such as H3K9me2/3, and heterochromatin proteins 1 (HP1). KDM4A is a member of the KDM4 subfamily of Jumonji lysine demethylases regulating H3K9 methylation, and binding H4K20me2/3. H4K20me3 is often lost in cancer cells whereas increased during senescence, and some studies suggest that it could localize in the SAHF. This epigenetic mark is also enriched at pericentric heterochromatin regions, where its deposition depends on the presence of H3K9me3 and HP1. In this work we demonstrate that H4K20me3 deposition in the SAHF depends on the previous formation of H3K9me3 foci, and that both heterochromatin marks contribute to the stabilization of RasV12-induced senescence. We propose that KDM4A alters the heterochromatin landscape of senescent cells by disrupting H3K9me3, HP1 and H4K20me3 foci, thus promoting senescence reversal and cellular transformation. We also performed siRNA-mediated depletion of previously reported H4K20 methyltransferases and identified NSD2, Suv420H1 and Suv420H2 as the enzymes implicated in H4K20me3 deposition in the SAHF. Our results suggest that histone lysine methylation plays a crucial role in regulating the long-term stability of OIS and expose the potential of inhibiting KDM4A to re-establish senescence-associated proliferation arrest in cancer cells.

A FRAMEWORK FOR SUMMARIZING CHROMATIN STATE ANNOTATIONS WITHIN AND IDENTIFYING DIFFERENTIAL ANNOTATIONS ACROSS GROUPS OF SAMPLES.

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Genome-wide maps of epigenetic modifications are powerful resources for non-coding genome annotation. Maps of multiple epigenetics marks have been integrated into sample-specific chromatin state annotations for many samples of various cell or tissue types. With the increasing availability of multiple chromatin state maps for biologically similar samples, there is a need for methods that can effectively (1) summarize the information about chromatin state annotations within groups of samples and (2) identify chromatin state differences across groups of samples at a high resolution.

To address this, we developed CSREP, which takes as input chromatin state annotations for a group of samples and then probabilistically estimates state assignment probabilities at each genomic position, which it then summarizes with the maximum-probability chromatin state at each position for the group. CSREP uses an ensemble of multi-class logistic regression classifiers to predict the chromatin state assignment of each sample given the state maps from all other samples with shared biological properties. The difference of CSREP's summary probability assignments for two groups can be used to identify genomic locations with differential chromatin state patterns.

Using groups of chromatin state maps of a diverse set of cell and tissue types, we demonstrate the advantages of using CSREP to summarize chromatin state maps. We show that CSREP is better able to predict the chromatin state of held out samples in a group than the counting-based baseline approach. We also show that CSREP can identify biologically relevant differences between groups at a high resolution, such as predicting cell-type-specific peaks of histone modification marks. The CSREP source code, and the summary chromatin state maps for 11 sample groups from Roadmap Epigenomics project, and 75 sample groups from Epimap is available at http://github.com/ernstlab/csrep.

METHYLATION QUANTITATIVE TRAIT LOCI (MQTLS) IN THE HUMAN PLACENTA

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Introduction. The placenta is an organ that mediates fetal growth. Placental dysfunction is implicated in various adverse pregnancy outcomes such as pre-eclampsia (PE) and fetal growth restriction (GFR). The polygenic nature of these complex diseases may have prevented genome-wide association studies from identifying reproducible genetic variants that fully explain the etiologies. An approach to enrich for loci functional inferences is to integrate genetic data with DNA methylation (DNAme) data. Identifying functionally important genetic variants in placentas through a methylation quantitative trait loci (mQTL) approach may better our understanding of genetic influences on pregnancy complications.
Objective. To identify genetic variants in the placenta that are associated with DNAme, and to explore their functional importance for healthy fetal development.
Method. SNP genotype and DNAme data of the placenta were obtained from two independent cohorts, a public dataset from the United States-based NICHD cohort, and one from Vancouver, BC. Data were collected using the Infinium Omni SNP array and Illumina Infinium HumanMethylation 450K array (or 850K array). Standard preprocessing and QC steps were applied, with the notable exception that we retained SNPs that deviated from Hardy-Weinberg equilibrium (HWE) as genotype frequencies may be due to population substructure rather than genotyping errors. Cis-mQTLs were identified via the MatrixeQTL package, using a window size of 300kb (+/- 150kb from the SNP).
Results. In the NICHD cohort (n = 289, all uncomplicated), 75,286 mQTLs involving 6,458 unique CpGs were identified (FDR<0.05, delta beta > 0.075), 38.5% of which were located on chromosome 6, which houses the highly polymorphic *HLA* genes. SNPs that deviated from HWE accounted for 2.78% the mQTL hits (2,091 mQTLs), and were retained in the analysis as the majority of the genotype frequencies were consistent with population substructure rather than genotyping error. mQTLs were enriched in enhancer regions and depleted in promoters. The top pathways associated with these loci included peptide antigen binding, cell periphery, ER membrane, plasma membrane, and MHC protein complex. In the Vancouver cohort (n = 11 FGR, 9 PE, 54 uncomplicated pregnancies), we replicated 32% of these hits (24,241 mQTLs).
Conclusion. Methylation quantitative trait loci (mQTLs) are widespread in human placentas. Genetic variants related to immune function, especially the *HLA* genes on chromosome 6, may play an important role in placental gene expression variation and in turn placental development. Future work will test the association of these loci with pregnancy outcomes.

NONCANONICAL REGULATION OF IMPRINTED GENE *IGF2* BY AMYLOID-BETA 1-42 IN ALZHEIMER'S DISEASE

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Reduced insulin-like growth factor 2 (IGF2) levels in Alzheimer's disease (AD) may be the mechanism relating age-related metabolic disorders to dementia. Since *Igf2* is an imprinted gene, we examined age and sex differences in the relationship between amyloid-beta 1-42 ($A\beta_{42}$) accumulation and epigenetic regulation of the *Igf2/H19* gene cluster in cerebrum, liver, and plasma of young and old male and female 5xFAD mice, in frontal cortex of male and female AD and non-AD patients, and in HEK293 cell cultures. We show IGF2 levels, *Igf2* expression, histone acetylation, and *H19* ICR methylation are lower in females than males. However, elevated $A\beta_{42}$ levels are associated with $A\beta_{42}$ binding to *Igf2* DMR2, increased DNA and histone methylation, and a reduction in *Igf2* expression and IGF2 levels in 5xFAD mice and AD patients, independent of *H19* ICR methylation. Cell culture results confirmed the binding of $A\beta_{42}$ to *Igf2* DMR2 increased DNA and histone methylation, and reduced *Igf2* expression. These results indicate an age- and sex-related causal relationship among $A\beta_{42}$ levels, epigenomic state, and *Igf2* expression in AD

and provide a potential mechanism for *Igf2* regulation in normal and pathological conditions, suggesting IGF2 levels may be a useful diagnostic biomarker for $A\beta_{42}$ targeted AD therapies.

MULTI-FACTOR CHIP-EXO REVEALS TF BINDING DYNAMICS WITHIN CONSERVED ORTHOLOGOUS CIS REGULATORY MODULES IN THE MAMMALIAN LIVER

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Cis-regulatory modules (CRMs) facilitate gene expression through the combinatorial recruitment of transcription factors (TFs). Previously, we used chromatin immunoprecipitation followed by sequencing (ChIP-seq) to map the genome-wide binding of 4 liver TFs (CEBPA, HNF4A, ONECUT1 and FOXA1) in 5 mammalian species (human, macaque, dog, mouse, and rat). We performed a multispecies analysis of liver CRMs which demonstrated that conserved orthologous combinatorial TF binding is a prominent feature of liver gene regulatory networks, and that conserved CRMs have been recurrently mutated in human diseases.
Here, we use ChIP-exonuclease (ChIP-exo) sequencing of the same TFs in mouse liver to study TF dynamics within conserved CRMs including cooperative binding, turnover, and retention of TF binding across evolutionary timescales. ChIP-exo footprinting analysis of CRMs revealed that co-binding preferentially occurs within short (<75bp) genomic distances but with little evidence for fixed-distance constraints between binding sites. Notably we find evidence for a FOXA1-ONECUT1 co-binding configuration that involves juxtaposed binding at a hybrid motif sequence.
To gain insight into TF binding dynamics within conserved orthologous CRMs we utilized a recently published deep learning method, BPNet, to predict TF binding events at single nucleotide resolution using our ChIP-exo data. Models trained on mouse liver ChIP-exo accurately predict ChIP-exo profiles in held-out mouse regions and as well as predict the regulatory impact of disease-causing regulatory mutations in blood coagulation and lipid regulating genes. We also observed motif 'micro-turnovers' (shifts, inversions) within a substantial fraction (10-15%) of conserved CRMs, including CRMs conserved in all five species. Overall, our results illustrate known and novel features of conserved combinatorial in vivo TF binding in mammals.

DNMT3A ROLE IN ADULT GLIOBLASTOMA

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DNA methylation is a key epigenetic modification, that is believed to drive carcinogenic changes. Surprisingly, a process of *de novo* DNA methylation, executed by DNMT3A proteins, that may be crucial for acquisition of oncogenic epigenetic changes, is poorly understood. Using TCGA ATACseq data we have verified that human glioblastoma (grade IV) cells more strongly activate DNMT3A2 isoform, when compared to lower grade gliomas (grade II, III). Gene expression level of DNMT3A is not different between grades II, III and IV, when TCGA repository was interrogated, but expression of DNMT3A2 isoform is higher than DNMT3A1 isoform, when glioblastoma tumors were compared to gliomas of lower grades. Next, we performed a differential gene expression analysis in TCGA data between glioblastoma with high or low DNMT3A2 isoform expression. DNMT3A2-high glioblastoma tumors were enriched for cell cycle related genes, putatively regulated by E2F family of transcription factors. We are currently investigating what may be the effect of DNMT3A2 expression on DNA methylation pattern.

ROLE OF UHRF1 BINDING TO H3K9ME3 IN DNAME MAINTENANCE AT HETEROCHROMATIC REGIONS IN NAÏVE EMBRYONIC STEM CELLS

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In mammals, DNA methylation (DNAme) primarily occurs at the 5th carbon of cytosine at CpG dinucleotides and is deposited by different DNA methyltransferases, with DNMT3A/3B responsible for *de novo* DNAme soon after the formation of the epiblast. Once established, DNAme is maintained by DNMT1, which is recruited to hemi-methylated DNA during replication. Interestingly, prior to the *de novo* methylation of the embryo, the genome undergoes a wave of epigenetic reprogramming, resulting in the erasure of most DNAme to confer totipotency on the embryonic stem cells (ESCs). This demethylation of the genome is largely a passive process and is the result of reduced DNTM1 recruitment to chromatin over successive rounds of cell division during preimplantation development. This decrease in DNMT1 function is mainly due to the inhibition of the enzyme, UHRF1, by the protein STELLA during this period. In the absence of Stella, UHRF1 functions to recruit DNMT1 to replication foci through its UBL domain and its deposition of H3K18/23-ubiquitin post-translational chromatin modifications, both of which are recognized by the RFTS domain of DNMT1. However, not all regions of the genome are demethylated during this wave of epigenetic reprogramming, with young transposable elements (TEs) and imprinted regions resisting the erasure of DNAme in the preimplantation embryo. Interestingly, these genomic regions tend to be enriched for H3K9me3, a heterochromatic mark, which has high affinity for the TTD domain of UHRF1. Thus, we propose that the residual levels of UHRF1, which escape inhibition by STELLA, are necessary for maintaining DNAme at H3K9me3-marked regions following DNAme erasure. To test this possibility, we will generate a mouse naïve ESC line with a knock-in construct encoding a non-functional TTD domain on one allele and a floxed WT *Uhrf1* on the other. Excision of the floxed *Uhrf1* will be induced using 4-hydrotamoxifen in the experimental ESCs. We will then determine whether disruption of the TTD domain results in loss of DNAme at H3K9me3-marked regions in ESCs. Targeted bisulphite sequencing will be performed on the ESCs to determine whether DNAme is reduced at specific TEs, and imprinted regions marked by H3K9me3. Whole genome bisulphite sequencing will then be carried out to determine the impact of the *Uhrf1* mutant

on DNAme genome-wide. Finally, to determine whether UHRF1 recruitment to H3K9me3-marked regions is perturbed by mutations in the TTD domain, we will perform ChIP-seq for H3K9me3 and UHRF1 in mutant ESCs. Ultimately, our study will further elucidate the mechanism by which DNAme is retained at critical regions of the genome during its erasure in preimplantation development.

FUNCTIONAL INTERACTION BETWEEN SFP1 AND NUA4 COMPLEX IN *SACCHAROMYCES CEREVISIAE* IN DIFFERENT GROWTH CONDITIONS

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Environmental changes affect ribosome biogenesis (RiBi) and ribosomal protein (RP) gene transcription in *Saccharomyces Cerevisiae.* Both glucose starvation and rapamycin cause the inhibition of the TOR pathway which is one of the major regulatory mechanisms of RiBi and RP gene expression. Being phosphorylated by TOR directly, Sfp1 is recruited to both genes' promoters in normal growth conditions. We found that Sfp1 could be acetylated at lysine 655 and lysine 657 by the only essential KAT complex – NuA4 in yeast. NuA4 is a 13-subunit lysine acetyltransferase complex that acetylates histone H4 and H2A dominantly. Its non-histone substrates have been discovered to be involved in many essential biological functions like DNA repair, autophagy and transcription. Co-immunoprecipitation experiments indicate that Sfp1 and NuA4 interact *in vivo*. Our previous ChIP-sequencing data confirmed NuA4 occupancy on RiBi and RP gene promoters. Sfp1 and NuA4 binding on these promoters decreases upon treatment with rapamycin – a TOR inhibitor.
GST-pull down assays show that Sfp1 can directly interact with NA4, leading to nucleosome acetylation on H4 and H2A lysines. We use the anchor-away technique to rapidly deplete Sfp1 and Esa1, the catalytic subunit of NuA4. ChIP-qPCR and CUT&RUN sequencing were used to investigate how NuA4 and Sfp1 affect the function of each other. Surprisingly, H4 acetylation deposited by NuA4 is not significantly affected by Sfp1 depletion on RiBi and RP gene promoters. Yeast mutants cells expressing Sfp1 with lysines 655 and 657 substituted to acetyl-mimic glutamine residues have growth defects when challenged with rapamycin. We observe morphological changes in mutants which may indicate cell cycle effects. Further studies are underway on how *Sfp1* mutants affect its function in different growth conditions, including glucose starvation. Other groups reported that Sfp1 binds in a glucose-regulated manner to the promoter of G1/S ("START") regulon genes. We speculate that Sfp1 acetylation is the glucose-sensing signal regulating its function through NuA4 activity. Genome-wide analysis of RNA polymerase II binding and NuA4 recruitment in wild type and mutant cells will be presented.

CHROMATIN PROGRAMMING AND REPROGRAMMING IN MOUSE GONOCYTE

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In mammals, epigenomic states such as DNA methylation, histone modifications, and chromatin accessibility regulate the transcriptional state of a given gene. These multiple epigenomic marks behave in a coordinated manner and affect the activity of genomic elements on chromosomes. But the detailed mechanism of this functional redundancy is not well understood because of the little good model systems in which multiple epigenetic marks change synchronously.
Mouse gonocytes, which are male germ cells from embryonic day 13.5 (E13.5) to postnatal day 3 (P3), undergo genome-wide *de novo* DNA methylation. We have previously revealed that transposable elements (TEs), which account for around 45% of the mammalian genome, are transiently derepressed during this period (Yamanaka et al., 2019, *Dev. Cell*). This "TE activation" has been proposed to make chromosomes to be amenable to *de novo* DNA methylation. At the same time, repressive histone marks, such as H3K9me3 and H3K27me3, are also reprogrammed globally. Thus, gonocytes are rare cell population where multiple epigenomic marks change synchronously
In this study, with new transcriptome dataset, we analyze the kinetics of host gene expression along with the gonocyte development. We found that meiotic genes that are normally upregulated in adult, are ectopically expressed in the embryonic stage. Furthermore, polycomb complex contributes to the formation of gonocyte transcriptome. In this meeting, we would like to discuss how multiple epigenomic marks sculpture the unique transcriptome of gonocyte and prepare for the adult testis development.

GENE REGULATORY MECHANISMS OF MOTOR NEURON DIFFERENTIATION BY A CHROMATIN REMODELING FACTOR BRM

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Chromatin remodeling plays an essential role in cell fate determination and differentiation. The BAF complex is one of the ATP-dependent chromatin remodeling complexes that has been reported to help modulate developmental processes. Brm is an ATPase subunit of the neuronal BAF complex and is vital for the BAF complex to modify chromatin architecture. *Brm* mutations cause an intellectual disability syndrome associated with decreased motor ability. The molecular mechanisms by which Brm regulates genomic targets during motor neuron development remain unclear. To examine this question, we generated *Brm* knockout mouse embryonic stem cells. We found that neural genes are down-regulated by *Brm* knockout in maturing motor neurons, whereas genes related to cell proliferation are up-regulated. We revealed that the absence of Brm causes an increased number of cells re-entering the cell cycle and therefore

contributes to the decreased cell-cycle exit profile. We also showed decreased differentiation rate in *Brm* knockout cells, suggesting Brm helps activate the expression of postmitotic neural genes by promoting cell cycle exit in neural progenitors. Using genomic mapping analyses, we demonstrated that Brm is required to activate neuronal enhancers, potentially bound by Onecut and Fox transcription factors. This study provides novel insights into the molecular mechanisms by which chromatin remodeling proteins regulate gene expression during motor neuron development.

PHENOTYPIC SEX AND SEX-CHROMOSOME COMPLEMENT IMPACT DNA METHYLOME INCLUDING BRAIN-SPECIFIC SEX-BIASED CAC METHYLOME

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Methylation of cytosines plays important roles in regulating transcription and chromatin structure. In humans, dysregulation of sex-biased DNA methylation, including CpH (non-CpG) methylation, has shown associations with neurodegenerative diseases, such as Rett syndrome. However, the mechanisms underlying sex-bias in CpH methylation remain unclear. To better understand the sex bias in CpH methylation and its contributing factors, we performed whole-genome bisulfite sequencing (WGBS) in mouse brain and identified sex-associated differentially methylated regions (sDMRs) for CpG dinucleotides and sex-associated differentially methylated cytosines (sDMCs) for CpH. We decoupled the effects of phenotypic sex and sex-chromosome complement by using mice with different combinations of sex-chromosome complement and gonadal sex. We identified sDMRs and sDMCs in three comparisons: sex reversed XY female mice (XY.F) vs XY males (XY.M) (reflecting the impact of gonadal sex), XY.F vs XX female mice (XX.F) (reflecting the impact of sex-chromosome complement), and XX.F vs XY.M (reflecting both factors). We also performed these analyses using livers of the same mice. Liver and brain CpG methylation data show that both phenotypic sex and sex-chromosome complement influence CpG methylation in brain and liver with sex phenotype having a major effect on autosomal CpG methylation levels in liver. In contrast, brain showed 4-11 fold more differential CpH methylation signals than liver, especially among CAC triplets. In brain, 1371, 1295, and 1433 CAC sDMCs were identified for comparisons XX.F vs XY.M, XY.F vs XY.M, and XX.F vs XY.F, respectively. Unlike CpG, only around 10% of CAC sDMCs were shared across comparisons. Interestingly, XY samples had higher methylation levels of X-linked CAC sDMCs in both comparisons of mice with different sex-chromosome complements: XX.F vs XY.M and XX.F vs XY.F. We next tested the possibility that CpG and CpH methylation were related and tested co-localization of sDMRs and sDMCs. Colocalization of CAC sDMC and CpG sDMR was observable on the X Chromosome (65% of sDMCs on average) but not on autosomes (3% on average). Our data suggest a complex regulation of sex bias in methylation with independent control of CpH methylation on autosomes and interplay between CpG and CpH methylations on the X Chromosome.

List of Attendees

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Roland	Sharen	Senior Client Manager	Génome Québec
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Safaei	Akram	Dr	University of Manitoba
Saini	Deepak	PhD Candidate	McGill University
Sattarifard	Hedieh	PhD Candidate	University of Manitoba
Sauvageau	Martin	Assistant Professor	IRCM
Sawchyn	Christina	PhD Candidate	Université de Montréal
Schulz	Marcel	Professor	Goethe University
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Shao	Xiaojian	Research Officer	National Research Council Canada
Shin	Jay	Group Leader	ASTAR/RIKEN
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Shooshtari	Parisa	Assistant Professor	Western University
Sihou	Djamouna	PhD Candidate	IRCM
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Singhal	Divya	PhD Candidate	University of Calgary
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Star	Gregory	Senior Customer Solutions Manager	QIAGEN
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Stickney	Cathy	Key Account Manager	Active Motif
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Tremblay	Lisa	Canadian Sales Manager	Illumina
Tsurumi	Haruko	Deputy Director	Japan Agency for Medical Research and Development Washington DC office
Turecki	Gustavo	Professor	Douglas Institute
Underhill	Alan	Associate Professor	University of Alberta
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Villeneuve	Tania	Sales Manager	MJSBioLynx
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Vu	Ly	Assistant Professor	University of British Columbia
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Wong	Martin	Graduate Student	University of British Columbia
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Yan	Xinyue	Student	EpigenDx, Inc.
Yeh	Ssu-Yu	PhD Candidate	University of Toronto
Yoshimi	Akihide	Principal Investigator	National Cancer Center Japan
Zhuang	Qinwei	Graduate Student	McGill University
Zia	Yass	Director	QIAGEN